A CLINICAL AND EXPERIMENTAL INVESTIGATION INTO THE EFFECTS OF OCCLUSIVE DRESSINGS ON WOUND CONTRACTION

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M.D.Thesis

University of Edinburgh

1992

DECLARATION

I formally declare that this thesis is based on my own original idea, has been carried out by myself after seeking the relevant specialist advice and has been written solely for presentation to the University of Edinburgh for the degree of Doctor of Medicine

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ACKNOWLEDGEMENTS

This thesis would not have been completed without the expert advice, facilities, and enthusiastic support given by the clinicians, scientists, and statisticians I have worked with whilst carrying out the work for this project. Professor D.A.McGrouther warrants special thanks for initially guiding me through my first paper and subsequently continuing to offer well balanced enthusiastic advice on all aspects of this thesis. Mr.Hackett and Mr.Walker at St Andrew's Hospital, Billericay, provided the initial encouragement to allow me to carry out the animal experiments. Sadly, Mike Hackett died before the project he enthusiastically supported could be finished.

I would like to acknowledge the excellent help of librarians Mrs Dunsford and Mr. Titley for their untiring work in carrying out "yet another" literary search.

I acknowledge that I am indebted to all the staff at the Smith and Nephew Research Establishment who taught me everything about the practicalities of setting up and running the animal experiments. Dr.I.Leigh and her helpful staff at the Experimental Dermatology Department of the London Hospital introduced me to the complex world of immunocytochemistry. Patricia Purkis, the senior technician gave considerable quantities of her busy time to help in the setting up and smooth running of the staining procedures.

Lastly, thanks to J. for her enthusiasm and advice, and also M. and K. for keeping everything in perspective.

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ABSTRACT OF THESIS (Regulation 3.5.10)

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Title of Thesis A Clinical and Experimental Investigation into the Effects of Occlusive Dressings on Wound Contraction.

No. of words in the main text of Thesis 45,000

Wound contraction is a component of normal wound healing which is often excessive in split skin grafted wounds. This manifests as extreme scarring which is responsible for the poor cosmetic and functional results seen when these skin grafts are used, for example, in the treatment of burns.

This thesis proposes to test the hypothesis that the contraction characteristics of different types of wounds can be affected when they are covered with an occlusive dressing. The work is divided into several sections, each studying a different type of wound.

The first study objectively assesses whether the effect of occlusive dressings on human open wound contraction is similar to that in published work using open wounds in rats.

In the second section a new animal model is created using bilateral split skin grafted wounds on a flexor and non-flexor surface of a rat. This model allows the measurement of:
A) the moisture vapour transmission from these wounds,
B) their surface areas and
C) the underlying myofibroblast cell numbers.
The use of in-animal control wounds reduces the number of animals required to produce data that can be analysed statistically.

The third study investigates the effect of an occlusive dressing on the contraction of human split skin grafted wounds. Patients with bilateral upper limb tattoos excised and split skin grafted will be studied. The surface area of one wound (occlusively dressed) will be compared to that of the conventionally dressed wound on the other limb.

The final section summarises and analyses the experimental results in the light of contemporary publications. Conclusions and suggestions for further studies are presented.

ABSTRACT

This thesis proposes to test the hypothesis that wound contraction is reduced when wound healing takes place in a moist environment.

In order to do so a series of experiments will be set up:

1) Human Study - Open Wounds

A human wound model would be set up to repeat previously published animal experiments. This model would examine the effect on wound contraction of dressing an open wound with an occlusive dressing.

2) Animal Study

An animal model would be created to allow a detailed investigation of the effects of dressing a split skin graft with an occlusive dressing.

This model would permit :

1) the precise rapid harvesting of split-skin grafts by a single operator
2) the application of split skin grafts to comparable wounds on a flexor and non-flexor surface on each side of the animal, on one side the skin grafts would be dressed with an occlusive dressing the opposite side would act as an in animal control. The dressings will be removed from different animal groups at weekly intervals up to 5 weeks to see if any effect the dressing has on wound contraction is related to the length of time the dressing is in situ.
3) the measurement of moisture loss from the skin grafted wounds on both sides of the animal. This will give an indication of the humidity of the wound healing environment.

4) the measurement of the surface area of the split-skin grafted wounds on the flexor and non-flexor surfaces. These measurements will be carried out on a regular basis up to 15 weeks after skin grafting. The measurement of surface area would quantify any effect a dressing had on the various phases of wound contraction

5) quantification of the myofibroblast cell population under all the split-skin grafts in the study. These cells are thought to be actively involved in wound contraction. Their numbers therefore, may be affected when wounds are covered with an occlusive dressing.

3) Human Study - Skin Grafted Wounds

A study would be carried out which would investigate the effect of covering a split skin grafted wound with an occlusive dressing and comparing it with a similar wound dressed conventionally. Two wounds per patient would be skin grafted and the graft surface areas measured at various time points up to 1 year. This would allow an accurate evaluation of any effect the dressing had on wound contraction.

If, by allowing split skin grafted wounds to heal in a moist environment subsequent wound contraction is reduced, then the cosmetic and functional results of burns therapy may be improved. This perhaps would allow an earlier return to a more productive role in society.

CONTENTS

Introduction and Aims of the Thesis

The stimulus for this work arose from the simple question; why does a wound covered with a split skin graft contract and what, if anything, can be done to reduce this phenomenon?

Split skin grafts are commonly used in burns surgery and the resultant scarring is both disfiguring and functionally restricting. A considerable amount of patient and hospital staff time is spent in attempting to reduce this unwanted complication. If, through early wound manipulation, this contraction could be reduced then the quality of patients lives would be enhanced and both patient and health service resources could be saved. (Perkins 1987)

BURNS

A burn injury is measured in terms of

a) the depth of the burn and

b) the percentage total body surface area involved.

A burn injury progressing beyond the deeper aspect of the dermis destroys the structures capable of allowing healing by re-epithelialisation. These wounds do not heal in a short period of time, therefore skin grafts are used to quickly provide an epithelialised barrier on the wound surface. This converts an open wound into a closed wound by providing a viable barrier to infection.

Mortality in burns is related to burn depth and the percentage body surface area burned. Early split skin graft coverage reduces the portal to infection and therefore the risk of septicaemia with its associated

mortality (Hendon 1989).

Once the burn wound is "healed" (ie covered with a viable epithelial barrier) attention then is turned to attempting to reduce wound contraction. The techniques employed to do so include long term physiotherapy, long term splintage or the wearing of cumbersome, expensive, custom made pressure garments. Pressure garments have to be worn for up to 23 hours per day for many months and many patients find them difficult to tolerate. Secondary surgery is often necessary to release contractures produced by the contraction of split skin grafted wounds.

WOUND CONTRACTION

Wound contraction is a phenomenon that occurs when open wounds heal by secondary intention (Grabb and Smith 1979). This phenomenon can result in complications which depending on the nature, site and extent of the injury can produce disfigurement, excessive scarring and impaired function of the affected organs (Peacock and Van Winkle 1970, Montandon et al 1973).

Wound contraction is reduced, but not eliminated when the wound is covered with a split skin graft. Covering the wound with a full thickness skin graft markedly reduces wound contraction (Padgett 1942) but for practical purposes, these grafts cannot be used to cover large defects produced for example in a burn injury.

When a split skin graft is used to cover a defect, wound contraction is most marked over the flexor aspect of a joint (Peacock and Van Winkle 1970, Grabb and Smith 1979) The cells thought to play an important role in wound contraction are myofibroblasts. These cells, described in

1971 by Gabbiani et al, have features of fibroblasts and smooth muscle cells. The numbers of these cells under skin grafts is thought to be influenced by the percentage of dermis within the graft (Rudolph 1979).

SYNTHETIC DRESSINGS

In animals, when one type of occlusive dressing is applied to an open wound, it has exactly the same effect as a full thickness skin graft in reducing wound contraction. This temporary dressing (Biobrane) consists of a nylon and silicone rubber sheet coated on one side with polypeptides from porcine dermal collagen. The dressing adheres to the wound in a similar manner to a skin graft by a polypeptide-fibrin clot interaction (Frank 1984).
If a permanent "dressing" could be made consisting of a split skin graft (instead of porcine dermal collagen) covered with a polyurethane occlusive dressing (instead of the nylon silicone bilayer) what effect would this have on the subsequent short and long term wound contraction?

AIMS OF THE THESIS

This study was therefore set up to investigate several parameters of wound healing under an occlusive dressing.
The study would be divided into two sections.
The first section would be concerned with repeating the animal work on open wounds in humans to see if a synthetic dressing had a similar effect in man as it had in the rat.
The second section would be concerned with:

a) a detailed animal investigation of several parameters of wound healing. Split skin grafted wounds on a flexor

and non-flexor surface would be covered with a synthetic dressing. These would be compared with conventionally dressed skin grafted wounds acting as controls on the opposite side of the animal.

b) a study in humans investigating the effect of an occlusive dressing on the contraction of split skin grafted wounds.

Patients with bilateral wounds requiring split skin grafts would have one skin graft covered with an occlusive dressing; the other would act as a conventionally dressed control.

Human Study - Open Wound Contraction

This initial study was undertaken using split skin graft donor sites to see if these wounds did contract and if this contraction was influenced in humans as it was in rats when the wound was dressed with a synthetic dressing. If this were the case then the findings of the second set of experiments could be more readily extrapolated to clinical practice.

Animal Study - Split Skin Grafts

This study was set up to observe, in the rat, the behaviour of split skin grafted wounds covered with an occlusive dressing. Different groups of rats would have split skin grafts occlusively dressed for different periods of time up to 5 weeks. These wounds would be compared with similar, conventionally dressed wounds on the opposite side of the animal.

The following parameters would then be measured:

Moisture Vapour Transmission

Moisture vapour transmission, measured with an

evaporimeter, would give an accurate tabulation of how the humidity of the split skin grafted wound was affected when covered with an occlusive dressing.

Surface Area

The surface areas of all wounds in the study would be measured. The areas of the grafts covered with a synthetic dressing would be compared with in animal control wounds, dressed conventionally.

Myofibroblast Cell Population

The split skin grafts would be covered with a synthetic dressing for periods up to 5 weeks. This time period was chosen for several reasons:

a) this is the active contraction phase of skin grafted wounds in rats, therefore if the dressing was to have an effect on wound contraction it should do so during this time.

b) Rudolph (1979) has shown, in rats, that myofibroblast numbers are present in very small numbers after this time. If these cells are responsible for wound contraction it can be assumed that any influence on their numbers would take place during this time.

c) the anti-actin antibody used to identify the myofibroblasts has been shown to be most successful during the phase of active wound contraction (Darby and Gabbiani 1990). The antibody allows light microscopy to be used, thus allowing larger tissue sections to be studied, giving a more accurate estimation of myofibroblast numbers in the tissue (McGrath 1982).

All the above measurements would be compared with in-animal control wounds which would be covered with a

split skin graft and then dressed conventionally.

Human Study - Split Skin Grafts

Patients who had bilateral upper limb defects requiring split skin grafts would be studied. The split skin graft on one limb would be covered with an occlusive dressing, the other would be dressed conventionally. Surface area measurements would be taken at operation and at various time points up to one year following surgery. The contraction of the skin grafted wounds on both limbs would then be compared.

Summary

This thesis proposes to study the effect a synthetic dressing has on several parameters of wound healing. Firstly, the original animal work will be repeated in humans to ensure the synthetic dressing effect is not species specific. Secondly, detailed studies will be carried out to see the effect, if any, synthetic dressings have on moisture vapour transmission, surface areas, and myofibroblast cell count under split skin grafts on a flexor and non-flexor surface of a rat.

A study in humans would then be carried out where the contraction of wounds covered with occlusively dressed split skin grafts would be compared with that of conventionally dressed identical grafts. Two wounds per patient would be studied to allow paired statistical comparisons. The length of time the skin graft was covered with the occlusive dressing would be influenced by the results of the animal study.

Statistics

The studies were designed to allow paired comparisons between wounds covered with an occlusive dressing and control wounds using Student "t" tests (Armitage and Berry 1987). Crude data would be collected using a microcomputer's spreadsheet facility. This software package would calculate the percentage change in all wounds with the original wound surface area acting as the 100% reference point.

Data would then be transferred to a statistics package where mean values, 95% confidence limits and paired or unpaired Student's t tests would be carried out.

CHAPTER ONE

Wound Healing

1.1 Wound Healing

1.2 Wound Contraction

1.3 Wound Dressings

1.4 Skin Grafts

1.5 Present Clinical Situation

1.1 Wound Healing

Overview

Wound healing takes place in an orderly manner with several phases occurring in a chronological sequence (Pessa et al 1987).

The first phase is one which occurs as an immediate non-specific reaction to wounding and is characterized by an acute inflammatory response. This usually lasts between 3-5 days (Peacock and Van Winkle 1970) if no wound contamination occurs (Clark 1988).

The phase following the acute inflammatory phase is the phase of fibroplasia - this term is used to denote the fibroblast and neomatrix components of the forming granulation tissue (Clark 1988). The phase of fibroplasia then progresses to the phase of collagen remodelling as the wound matures (Ross 1968).

Wound contraction takes place mainly during the phase of fibroplasia (Clark 1988) which lasts between 2-4 weeks in a rat and up to 1 year in a human (Baur, Parks and Larson 1977).

The time scale and magnitude of the latter two phases varies according to the species of animal studied and to the extent of the inflammatory response created by the initial tissue damage (Narayanan et al 1989).

Wound Healing - The Inflammatory Response

When an open wound is created the epidermal and dermal layers of the skin are disrupted. Tsuboi (1990) argued that this initial mechanical cell damage must be converted into chemical signals in order to cause cell

proliferation. He demonstrated that contents released from damaged cells, may, by increasing local fibroblast mRNA levels, act as initiating factors triggering the proliferation of surrounding undamaged cells.

Bleeding takes place from severed vessels and so the wound surface is covered initially with cells originating from the circulating blood. The local microvasculature becomes leaky to plasma proteins either as a result of the initial trauma or in reaction to local mediators (Dvorak, Kaplan, and Clark 1988).

Plasma constituents then extravasate into the interstitial tissue and clotting is initiated as a result of the activation of 3 systems;

1) the Extrinsic Pathway which is the main source of clotting
2) the Intrinsic Pathway which also activates bradykinin, therefore increasing vascular permeability
3) platelet activation which plays an important role especially when blood vessel disruption occurs (Dvorak, Kaplan, and Clark 1988).

The fibrin clot produced subsequently becomes the vascular collagenous matrix known as granulation tissue (Dvorak, Kaplan, and Clark 1988).

Many factors including;

bacterial endotoxin (Wahl et al 1974)

the products of the coagulation pathway (Kay 1974, Grotendorst 1984),

the presence of platelets covered with fibronectin (Wagner 1985),

various platelet derived cytokines (Deuel 1982 Wahl 1987)

the products of the fibrinolysis and kallikrein\kinin systems (Kaplan 1973 and 1972),
the activation of the complement cascade with the formation of C5a (Snyderman et al 1970),
and the presence of surface bacteria (Ward et al 1968, Schiffman et al 1975)
stimulate the migration of neutrophils and monocytes into the wound thus initiating the cellular aspect of the acute inflammatory response.

There are several cell types playing important roles in the inflammatory phase of wound healing

Platelets

Morphology

Platelets are anucleate discoid fragments approximately 2 micrometres in diameter, derived from bone marrow megakaryocytes as fragments budding off from the peripheral cytoplasm (Terkeltaub and Ginsberg 1988).
They contain at least three types of organelles involved in wound healing (Terkeltaub and Ginsberg 1988).
Alpha-granules store several platelet specific proteins and glycoproteins which will be discussed later.
Dense bodies are less numerous than alpha-granules, they contain serotonin, adenine nucleotides, calcium and pyrophosphate.
Lysosymes contain neutral and acid hydrolases.

Activation

Platelets can be activated by a variety of factors present in the wound environment. These include thrombin, A.D.P.,

arachidonic derivatives, exposed subendothelial collagen, immune aggregates, microorganisms, double and single stranded D.N.A., Complement and some bacterial lipopolysaccharides (reviewed by Terkeltaub and Ginsberg 1988).

Actions

Once activated, platelets release substances which have a profound influence on their environment.

Thromboxanes and prostaglandins affect local vascular tone and neutrophil adherence and function (Terkeltaub and Ginsberg 1988). Serotonin, released from the platelet dense bodies, acts as a vasoconstrictor, increases vascular permeability and is fibrogenic in nature (daPrada et al 1981). Thrombospondin, released from the alpha-granules, inhibits fibrinolysis and binds to matrix constituents (Leung 1984).

The alpha-granules also contain platelet derived growth factor, transforming growth factors alpha and beta, epidermal growth factor, and platelet factor 4 (a protein that neutralizes heparin in vivo). These factors have been found to have a significant influence on vascular permeability, leucocyte accumulation, fibroplasia, collagen synthesis and angiogenesis (Terkeltaub and Ginsberg 1988).

Polymorphonuclear Leukocytes

The polymorphonuclear leucocyte responds to inflammatory stimuli in hours to days, whereas the macrophage response takes days to weeks. This is because large reserves of leukocytes are stored in the bone marrow and are released

as a response to the trauma of wounding whereas a lag phase occurs because new pools of monocytes are generated as a response to these stimuli (Rappolee and Werb 1988). Therefore, the predominant cell in the wound environment in the initial phase of healing is the polymorphonuclear leucocyte. It marginates on capillary and venular endothelium and then migrates into the wound space (Knighton 1989). This process (diapedesis) was first observed in tadpole tails by Dutrochet in 1824 and in 1893 Metchnikoff proposed that this action was a response to chemotactic attraction from outside the vessel wall (Tonnesen, Worthen and Johnston 1988). The polymorphonuclear leucocyte is responsible for the initial destruction of contaminating bacteria and early wound debridement (Ross 1972, Falcone 1990).

It's response is relatively simple:

Inflammatory signals induce cell adhesion and chemoattraction, phagocytosis and bacterial killing occurs following the activation of an intracellular respiratory burst. Degranulation then occurs which releases hydrolytic enzymes and antimicrobial polypeptides (Tonnesen, Worthen and Johnston 1988). These products include PMN elastase, Myeloperoxidase, Lysosomal acid hydrolases, PMN collagenase, Lactoferrin, Defensins, Plasminogen activator, Reactive oxygen metabolites, active lipid metabolites and fibronectin (Rappolee and Werb 1988). These chemicals also destroy bacteria and may potentiate tissue injury and prolong the acute inflammatory response (Tonnesen, Worthen and Johnston 1988).

The acute inflammatory response wains probably when the balance of inhibitory mediators is greater than the stimulator ones. It is probable that the cessation of neutrophil influx varies with the initial wounding stimulus and injury site. Intact senescent neutrophils are probably phagocytosed by activated macrophages so reducing the cellular aspect of the acute inflammatory response by removing neutrophils from the wound without disrupting their cell membrane and causing further tissue damage (Haslett and Henson 1988).

Masure and Opdenakker (1989) state that, as leucocytes invade an area of inflammation they, like macrophages, lymphocytes and platelets, release cytokines.

Cytokines

Since their discovery there has been over 12,000 publications, numerous reviews and several books written about cytokines (Leutz and Graf 1990).

Coverage of the whole topic is not the remit of this thesis therefore a brief review of the articles relevant to wound contraction follows.

Cytokines are glycopeptide signal mediators which generally act at the site of production and influence cell migration (McGrath 1990) and proliferation (Masure 1989). Recent advances in biochemical and analytical technology have allowed the purification of these growth factors. Recombinant DNA techniques have meant that sufficient quantities of cytokines can be produced for experimentation so expanding the data on such peptides, indicating they are involved in tumour and fetal growth processes as well as wound healing (McGrath 1990).

Cytokines can regulate the release of microbicidal and cytocidal proteases by either the down- or up- regulation of the genes responsible for their production (Stanley 1988).

The effects of cytokines are not totally confined to the immediate wound environment. They can act on distant tissues inducing fever, endocrine changes, leucocytosis and synthesis of the acute phase proteins (Whicher and Evans 1990).

There are several ways in which cytokines or growth factors can get to a wound

Some growth factors (eg. insulin like growth factor I) are transported attached to a large molecular weight carrier protein in plasma and thus are delivered in an endocrine fashion.

Other cytokines (eg. platelet derived growth factor) are secreted by one cell and used by another local cell. This is a paracrine delivery. An autocrine system is when the cell secretes and uses the same growth factor (eg fibroblast somatomedin - Sm-C). Autocrine factors regulate local homeostasis by acting in positive and negative feedback mechanisms (McGrath 1990).

Growth factors are classified as either competence or progression factors. A competence factor (eg PDGF) accelerates the cell to the first growth arrest point, whereas a progression factor (eg Sm-C) stimulates the cell through the growth cycle committing it to DNA synthesis. Both types of factors are strongly synergistic (McGrath 1990).

Target cell membrane receptors show specific and high

affinity binding properties. Activation of these receptors elicits a rapid sequence of biochemical events within the cell. Among these are the activation of tyrosine kinase activity and the transcription of the proto-oncogenes c-fos and c-myc (Leutz and Graf 1990). The increased expression of these two proto-oncogenes is necessary for the mitogenic response of target cells to growth factors (Coughlin, Escobedo and Williams 1988)

Cytokines have also been found to regulate the movement (Postlethwaite 1976), and metabolic activities (Postlethwaite 1988) of fibroblasts. This will be further discussed in later sections.

Lastly, recent studies showing the beneficial wound healing effects of platelet derived fluid (Knighton et al 1990) and a purified growth factor (Brown et al 1989) indicate that wound manipulation with cytokines may become a recognised method of treating some wounds in the near future (Hunt and La Van 1989).

The Macrophage

As wound healing progresses the mononuclear phagocyte becomes the predominant cell. After 2-3 days, macrophages begin to appear in the wound and become the predominant phagocytic cell of the inflammatory tissue. They appear in the wound from several sources; from the resident tissue, from the circulating blood shed at the time of the original injury (Remensnyder 1982), and (mainly) from the bone marrow as a response to factors released during the wound healing process (Stewart et al 1981). When the circulating monocyte leaves the blood it differentiates

into a specialized tissue monocyte or macrophage (Clark 1988).

Macrophage Activation

Macrophage accumulation and activation is probably stimulated by various chemoattractants found in the wound. These include inflammatory reactants such as leukotrienes and complement split products (Rappolee and Werb 1988) collagen fragments - especially type I (dermal) collagen, elastin, fibronectin and enzymactically active thrombin (Clark 1988), bacterial derived factors (Riches 1988) as well as the cytokines previously mentioned.

The migration of the macrophage (and several days later the lymphocyte) is probably regulated in part by dermal vascular endothelium in the wound locality. Cai et al (1990) found that mononuclear cell-vascular endothelial adhesion (the precursor to diapedesis) was maximal several days after polymorphonuclear adhesion.

Macrophage Function

Recent studies have shown that macrophages occupy a central position in the transition between wound inflammation and fibroplasia (Clark 1988) and the regulation of wound repair (Diegelmann 1981).

Firstly, they carry out phagocytosis, so assisting neutrophils in the defence against bacteria. Secondly, it has been shown that macrophages release vasoactive mediators, chemotactic factors, enzymes (Clark 1988) and cytokines which influence the formation and maintenance of granulation tissue (Knighton and Fiegel 1989).

Macrophage function is related to its state of differentiation and\or activation (Fukasawa et al 1988).

The phagocytosing of cell constituents (eg. lipid) by macrophages results in the subsequent release of transforming growth factor-alpha and interleukin-1. These cytokines stimulate angiogenesis, epidermal regrowth and granulation tissue formation. Transforming growth factor-beta, produced by activated macrophages, is itself a macrophage and fibroblast chemoattractant (Rappolee and Werb 1988).

Johnson (1976), Postlethwaite (1976), Littman (1977) and Wahl (1978) have all described numerous soluble factors produced by lymphocytes (lymphokines) that can activate macrophages.

Other factors with macrophage stimulating effects include immune complexes and the complement cleavage products C3b (Allison 1978), C5a (Grotendorst 1984) and C567 (Cederna 1988).

Secretory Products

The macrophage, through its secreted products, can have a profound influence on the behaviour of surrounding cells (Korn 1980). Nathan in 1987, listed some 87 macrophage secretory products which had known effects on T and B lymphocytes, natural killer cells, macrophages, neutrophils, fibroblasts, eosinophils, endothelial cells, synoviocytes, chondrocytes, osteoblasts, osteoclasts, adipocytes, skeletal myocytes and various other mesodermal cell types present in a healing wound.

The macrophage can carry out a variety of specialized responses to surrounding stimuli.

These include:

increased lysosomal activity (Morland 1977),

increased secretion of proteinases (Wahl 1974), the releasing of complement components (Littman 1977), increased capacity to inhibit cell proliferation (Baird 1977), increased production of interferon (Machara 1977), secretion of endogenous pyrogen (Bodel 1977), formation of tissue thromboplastin (Allison 1978) and increased prostaglandin synthesis (Humes 1977). Indeed, Rappolee and Werb (1988) state that macrophages have the potential to mediate the five temporal segment responses to immunological challenge (inflammation, killing, immunity, debridement and wound healing). Activated macrophages can also secrete a factor that stimulates fibroblast proliferation and fibroblast D.N.A. synthesis at low levels and inhibits these functions at high concentrations (Diegelmann 1979). Turck (1987) described the effects on fibroblast function of 10 cytokines secreted by macrophages indicating perhaps the influence this cell has on fibroplasia. The secretion of collagenase by fibroblasts, for example, appears to be an inducible de novo protein synthesis tightly controlled by factors secreted by activated macrophages (Riches 1988). Leibovich and Ross (1975) have shown that, without this cell subsequent wound debridement and fibrosis is retarded.

Lymphocytes

In addition to the highly specific (single cell type target) factors produced by macrophages, fibroblasts are also stimulated by less specific factors produced by

T-lymphocytes (Schreiber 1985) present in the environment of a healing wound.

Efron et al (1990) suggest from their studies using monoclonal antibodies that a specific subset of T-lymphocytes stimulates wound fibrosis in mice. It would seem that T-lymphocytes are recruited into the healing wound several days later than monocytes, possibly attracted there by factors in the dermis remaining in or around the wound after the initial injury (Cai et al 1990). Johnson and Ziff (1976) and Wahl et al (1978) found that stimulated T-lymphocytes produce factors which increase fibroblast proliferation and collagen production. Fibroblast prostaglandin synthesis was also increased which Wahl et at postulated served as a negative feedback mechanism.

Although lacking an objective method of accurately counting cell numbers Martin and Muir (1990), looked at human wounds and found that T-lymphocytes were present in scars up to three years old. This indicated that perhaps the T-lymphocyte influences the late phase of normal and abnormal scar remodelling perhaps more so than the macrophage.

<u>Wound Healing - Fibroplasia and Collagen Remodelling</u>

Following the initial acute inflammatory response, the original fibrin clot matures into granulation tissue (Clark 1988).

This can be regarded as having two zones (Remensnyder 1982), a superficial zone of red inflammatory tissue and a deeper zone of fibrotic tissue.

The superficial zone is produced as a result of the initial and continuing inflammatory response. Initially, it consists of cells which have been shed into the area of injury and this area becomes predominantly populated by neutrophils. It has a high concentration of very permeable vascular channels which allow fluid to leak into the wound so providing a dessication barrier to the outside environment.

As the granulating wound matures the deeper zone becomes more defined. This fibrotic zone provides wound strength and is associated with collagen metabolism and wound contraction. Endothelial cells, macrophages and various types of fibroblasts are the predominant cells present in this compartment. Endothelial cells appear within 24-48 hours after wounding as vascular buds. Fibroblasts appear 4-5 days after wounding and appear to synthesize the molecules of the extracellular matrix in the same order as they do in embryonic development and tissue culture (Nicoletis 1977). Glycosaminoglycans are manufactured first, followed by collagen synthesis. It is interesting to note that proteoglycans are intimately connected with the tips of growing capillary buds, probably linking migrating endothelial cells to the extracellular matrix and helping to organize the capillary basal lamina (Ausprunk 1981).

Collagen synthesis is concentrated in this zone with the fibroblasts producing procollagen elements intracellularly. These elements are secreted and aggregate into fibrils of mature collagen in the extracellular matrix. In the early stages of wound healing the embryonic

type III collagen predominates but this is almost completely replaced by type I collagen as the wound enters the later phases of healing. (Remensnyder 1982)

The collagen continues to undergo remodeling as wound healing progresses. Collagen degradation is carried out by macrophages (Wahl 1974). Baur et al (1979) have described "fibroclasts" and "myofibroclasts". These cell types were found in human granulation tissue and scars. They were identical to fibroblasts and/or myofibroblasts with respect to size, shape, orientation and distribution but they also contained collagen fibrils within cytoplasmic vacuoles. This indicated that external collagen had been phagocytosed as newly formed collagen in fibroblast cytoplasmic vesicles is in the form of procollagen prior to its exocytosis. Baur further postulated that these cell types were responsible for collagen remodeling.

As the wound matures collagen metabolism continues and this results in a strong supportive base providing structural integrity to the wound.

The open wound is also contracting and when the deeper zone is covered by the superficial zone (ie.the wound is open) the stimulus to wound contraction exists. This stimulus is reduced when the superficial zone is finally covered with epithelium ie. the wound is said to be healed (Remensnyder 1982).

When the superficial zone is excised and the deeper zone is covered with a skin graft this stimulus may still be present but it is probably influenced by the type of skin graft used. Covering a wound with a full thickness skin graft reduces water loss from the wound (Lamke 1971) and

may reduce its contraction by altering the numbers of myofibroblasts within the wound (Rudolph 1979).

Epithelialisation

Epithelial repair begins shortly after clot formation when an open wound is created. Migration of residual epithelium over viable tissue and under formed scab takes place after a lag period (Stenn and Depalma 1988). Epithelium covers the wound by a process of active horizontal movement rather than growth pressure from behind the advancing edge (Pang et al 1978). The lowest cells of the stratum spinosum are the first cells to begin migration. They loose desmosomal and hemidesmosomal contacts, the basement membrane zone loses definition and the cells become less firmly attached (Stenn and Depalma 1988). Microfilament bundles, staining with antiactin and antimyosin antibodies, appear in the periphery of these cells. This coincides with an increase in the proportion of the cell surface occupied by gap junctions, suggesting a mechanism of synchronized locomotion (Rungger-Brandle and Gabbiani 1983). The leading edge cells become actively phagocytotic which may help cell migration which may be enhanced by the presence of fibronectin (Takashima and Grinnell 1984). Mitosis takes place approximately 1 mm behind the advancing edge and appears to be diurnal in nature with the greatest mitotic rates occurring during rest and sleep (Baur, Parks and Larson 1977).

Behind the zone of mitosis the epithelium is undergoing stratification as cell division increases the thickness of this structure (Peacock and Van Winkle 1970).

The cell sheets continue to advance until the microvillus processes from opposing epithelial cells touch each other and contact inhibition of movement occurs. This results in the cells losing the phagocytic vacuoles and the actin and myosin cytoplasmic microfilaments. The numbers of desmosomes and hemidesmosomes increase, tonofilament bundles reattach to the cell periphery and the basement membrane reforms (Stenn and Depalma 1988).

Following the restoration of epithelial continuity, mitosis commences at the junction of the two epithelial sheets so thickening the newly formed epidermal layer (Krawczyk 1971).

The rate of epithelialisation is enhanced if the wound is allowed to heal in a moist environment (Winter 1972, Alvarez 1983, Eaglstein 1988).

1.2 Wound Contraction

Wound contraction has been defined as "diminution in size of an open wound which is the result of the centripetal movement of the whole thickness of the surrounding skin". (Van Winkle 1967)

It has been known for some time that granulation tissue is responsible for wound contraction (Pollock, Editorial, Lancet 1870). Abercrombie (1956) reported that the collagen fibres within the granulation tissue cannot themselves contract so attention was turned to the fibroblast (James D.W. 1969).

Fibroblasts

The Stimulation of Fibroblasts

The polymorphonuclear neutrophil was thought to play an important role in wound repair with many authors noting its presence in the early phases of wound healing (Page 1958, Ward 1970). Carrel (1924) assumed that these cells produced "trephones" which regulated subsequent fibroplasia. However, Ross and Benditt (1961) found that fibroblasts appeared in wounds some 4 days after neutrophils indicating that the latter cells did not have an immediate effect on fibrosis. This theory was further substantiated by studies from Simpson and Ross (1972) which showed that wounds in neutropenic animals healed normally. It would seem that neutrophils are necessary for the removal of bacteria and foreign debris but are not directly responsible for the regulation of fibroplasia and wound repair.

Ross and Benditt also observed that macrophage

infiltration always precedes fibroplasia. Animals made monocytopenic by the administration of hydrocortisone and anti-monocyte serum exhibited significant delays in the appearance of fibroblasts and the deposition of collagen in open wounds. Observations since then by several authors suggest that mononuclear cells and macrophages play an important role in the removal of tissue debris (Martin et al 1988) and the regulation of collagen metabolism (Viljanto and Raekallio 1976, Wahl 1978, Diegelman et al 1981, Hibbs 1983, Dohlman 1985). Turck (1987) reviewed the regulation of fibroplasia and found that many polypeptides stimulate the migration, proliferation and synthetic functions of fibroblasts. These factors are different to the macrophage and neutrophil chemoattractants (Grotendorst 1984). It would seem that specific surface receptors on the fibroblast bind the attractant molecules which, in some way, stimulate the cell to move towards the chemoattractant (Schiffmann 1979).

Princolo et al (1990) have found that as a wound matures the fluid produced has a declining ability to stimulate fibroblast proliferation. They found that after 10 days, fluid from a rat wound reduced fibroblast proliferation without altering collagen production. Stimulatory activity of the wound fluid was found in the >300 kDa. molecular weight fraction whereas the inhibitory activities of the wound fluid were confined to the <10 kDa. molecular weight band. In this study the possibility of a carrier macromolecule artificially increasing the molecular weights could not be excluded but these findings are similar to other studies where factors were identified in

wound fluid that influenced the behaviour of specific cells (Roberts 1987).

Cytokines

Platelets are one of the first cell types to arrive at the wound site. These cells contain a potent chemoattractant for fibroblasts (Seppa 1982). Platelet derived growth factor (PDGF) stimulates the migration of fibroblasts at low concentrations and, at higher concentrations stimulates fibroblast proliferation (Grotendorst 1984). PDGF also has a synergistic effect with insulin-like growth factor and epidermal growth factor which may be important in the modulation of the wound healing process (Lynch et al 1987).

Platelets also release transforming growth factor beta (TGF-beta)(so called because of its ability to induce phenotypic transformation in normal rat fibroblasts) which is stored in the alpha granules in similar concentrations to PDGF. It is released during platelet degranulation at the time of injury in an inactive form. It is then activated by enzyme activity or by the acidic environment associated with acute inflammation (Ksander et al 1990). The actions of TGF-beta are synergistic with PDGF and Epidermal Growth Factor (EGF) - two cytokines also found in platelets (Assoian 1988).

TGF-beta is also found in activated macrophages, lymphocytes and fibroblasts (Pierce et al 1989).

Its actions include:

monocyte chemotaxis (Wahl S.M.et al 1987)

the stimulation of monocyte production of interleukin-1

(Wahl S.M. et al 1987),
fibroblast chemotaxis (Ksander et al 1990)
the alteration of fibroblast phenotype (Roberts et al 1988)
the induction of fibrosis in the adult and fetal wound (Krummel et al 1988),
increased collagen (Narayanan et al 1989, Pierce et al 1989), fibronectin (Roberts et al 1988) and glycosaminoglycan (Hebda 1988) production,
the enhancement of connective tissue formation and open wound strength (Ksander et al 1990)
accelerated tensile strength of incisional wounds (Brown et al 1988)
the stimulation of protease inhibitors and the inhibition of fibroblast protease production (Sporn et al 1987)
increased epidermal migration (Hebda 1988)
regulation of local vascular tone (Kurihara 1989).
the stimulation of collagen matrix contraction by fibroblasts (Montesano and Orci 1988)
The above strongly suggest that TGF-beta has an important role as an endogenous mediator of wound repair and fibrosis (Assoian 1988, Ksander et al 1990).

In 1983 Postlethwaite and Kang found that polypeptides between 10,000 and 60,000 daltons produced by human monocytes enhanced the proliferation of cultured fibroblasts as assessed by the uptake of H^3thymidine. Since then factors identified as originating from monocytes which activate fibroblasts include interleukin 1 (IL-1)(Schmidt 1984), tumour necrosis factor (Vilcek 1986)

and platelet derived growth factor-like factor (Martinet 1986). Macrophages also release factors with similar activities to IL-1, tumour necrosis factor and fibroblast growth factor. (Dohlman 1984, 1985)

Interactions between fibroblasts and mediators may be selective, for example the C1 component of complement is mitogenic to a sub population of fibroblasts and prostaglandin E2 and some products of activated macrophages affect growth and collagen synthesis of only some fibroblasts (Narayanan et al 1989). Russell et al (1988) have shown that fibroblasts from keloid scars can respond to lower growth factor concentrations than normal fibroblasts indicating the variety of target cell responses to these polypeptides.

It would seem that the concentrations (Narayana et al 1989) and interactions (Lynch et al 1987) of different cytokines at differing stages of wound repair are likely to determine the length and intensity of the various phases of wound healing. This theory is supported by Broadley et al (1989) who found that the reduction of cytokine concentrations by a specific neutralizing antibody can have a significant reduction on subsequent wound healing indices.

McGrath (1990) aptly describes growth factors and their receptor cells as individual symbols which have little meaning by themselves. The meaning of each individual cytokine can only be understood within the total context of all the other signals operating on the cell. The effective integration of the many different cell types and

cytokines results in coordinated wound repair whereas the loss of this integration results in activities not dissimilar to carcinogenesis (McGrath 1990).

Summary

It is possible that fibroblast migration and proliferation is stimulated by the many cells present in the initial phases of wound healing. Platelets (Pierce et at 1989), macrophages (Knighton 1989) and lymphocytes (Wahl 1978) all release products that stimulate fibroblast numbers and function until a certain cell density is reached (Tsubio 1990). At that point, proliferation is reduced possibly by paracrine or autocrine mediators (Korn 1980, Sharma 1986, Freundlich 1986) while collagen production continues unaltered (Pricolo 1990).

Myofibroblasts

Eosinophilic stellate cells in healing wounds had been noted by pathologists for many years. Their size and shape indicated that they were fibroblasts but they lacked the cytoplasmic basophilia associated with collagen synthesis (Anonymous, Lancet 1978). In 1971 Gabbiani noted that these cells had contractile properties and histological features similar to smooth muscle cells and fibroblasts respectively, he therefore called them myofibroblasts. Since then evidence has continued to accumulate indicating that these cells are actively involved in wound contraction.

Morphology

The electron microscopic appearance of myofibroblasts is characteristic (Gabbiani 1971, Rudolph 1978, Ghadially 1982). They have some of the features of fibroblasts and smooth muscle cells combined. Under the electron microscope fibroblasts are long, flat cells surrounded by collagen.

Fibroblasts produce protein (collagen), therefore they have a prominence of rough endoplasmic reticulum as well as large numbers of mitochondria both within the cell body and in the long peripheral processes.

Myofibroblasts have three additional features more in keeping with smooth muscle cells (Montandon and Gabbiani 1973).

1) Cytoplasmic Microfilaments

Cytoplasmic microfilaments are classified according to their width (Ghadially 1982).

Thin filaments (4-7nm thick) contain actin,

intermediate filaments (6-12nm thick) of which their are 5 types are generally thought to be cytoskeletal in nature, and thick filaments (11-16nm thick) contain myosin. Filaments found in myofibroblasts are the same thickness as filaments in smooth muscle cells. They are 4 to 8 nm thick and are usually arranged in a manner parallel to the longitudinal axis of the cell. They fill much of the cytoplasmic space and have many electron-dense areas scattered within the microfilament bundles or beneath the plasmalemma (Rungger-Brandle 1983). This is similar to the attachment sites in smooth muscle cells (Guber 1978). These fibres contain the same proteins (actin, myosin, alpha-actinin and tropomyosin) arranged in a pattern similar to that seen in sarcomeres (Burridge 1981). They are predominant in non-motile fibroblasts and myofibroblasts (Rungger-Brandle 1983). Burridge (1981) has observed that these microfilaments or stress fibres are formed when a cell becomes adherent to the surrounding structures. Large microfilament bundles form parallel to the direction of cell contraction. Although some shortening occurs, the contraction of the filaments is mainly isometric in nature, since once the cell contracts its shape remains unchanged for long periods of time. When the adhesion to the surrounding substrate is released the fibres and the cells relax (Burridge 1981).

Repeated work by Gabbiani and his co-workers (most recent 1990) has shown that the microfilaments within myofibroblasts can be stained with the anti-actin monoclonal antibody anti-alpha-sm-1, the stain used in one section of this thesis.

2) Nuclear Membrane Deformities

When smooth muscle cells contract their nuclei become distorted. Fibroblasts have smooth, usually oval, nuclei whereas myofibroblasts have nuclei with multiple deep indentations similar to the nuclei of contracting cells (Majno et al 1971).

3) Surface Differentiations

Fibroblasts do not attach themselves to each other or the surrounding stroma but myofibroblasts do (Guber 1978, Doillon 1987). There are two types of surface differentiations which allow this attachment (Gabbiani 1971):

a) macula adherens or desmosomes are dense plates on the cell membrane, allowing intercellular connections
b) hemidesmosomes on the cell membrane enable the cell to attach itself to the surrounding stroma.

Doillon et al (1987) postulate that there could be three ways in which the myofibroblast could induce wound contraction. Cell to cell adhesions, cell to extracellular matrix adhesions possibly by an anchoring strand (Baur and Parks 1983) or a combination of both would allow these cells to become anchored to the wound substance. This anchoring may trigger the isotonic and isometric contraction (Burridge 1981) of the intracellular, actin based microfilaments (Gabbiani 1990), inducing cell contraction resulting in overall wound contraction.

Distribution

Healing Wounds

Myofibroblasts were first identified in the granulation tissue of healing wounds (Majno et al 1971). As early as

six days post wounding, fibroblasts with the electron microscopic characteristics of myofibroblasts were identified (Darby 1990) in rat open wounds. These fibroblasts stained positive with anti-alpha-sm-1 antibody indicating the presence of microfilaments containing actin in their cytoplasm.

Other Sites

Myofibroblasts have also been reported in many different pathological and physiological situations (Guber 1978). Ghadially in 1982 reviewed the literature and found evidence of the identification of myofibroblasts in the palmar nodules of Dupuytren's contracture, intrinsic fibrosis of muscle, ganglia, fibrous capsules around breast prostheses, atherosclerotic lesions, cirrhotic liver, injured human menisci, pseudointima of dacron vascular prostheses, desmoid fibromatosis, plexiform tumour of the uterus, nodular fasciitis, tendon sheath and infantile digital fibromas, congenital generalized fibromatosis, non-ossifying fibroma of bone, giant cell granuloma of the jaw, malignant fibrous histiocytoma, Hodgkins lymphoma, epitheliod sarcoma, and the stroma of various carcinomata including breast carcinoma, follicular carcinoma of the thyroid, colonic carcinoma and basal cell carcinoma.

Myofibroblasts have also been identified in non-pathological tissues. In the gut, they are thought to regulate the vascular and stromal spaces in the villus (Guldner 1972), in the lung they have been implicated in the control of ventilation (Kapanci 1990). They have been

identified in the seminiferous tubules of the human testis, the testicular capsule of the rat, the theca externa of ovarian follicles, the adrenal capsule, the renal capsule of mammals, mesenteric lymph nodes and human fetal spleen. Myofibroblasts have been shown to be virtually the only cell present in Wharton's jelly in the umbilical cord indicating that these cells have a dual function of secretion and contraction in this particular tissue (Ghadially 1982).

They have been found in areas where active contraction is occurring eg. hypertrophic scars (Baur 1975, Eddy 1988), fibromatic lesions (particularly the nodules in Dupuytren's disease)(Vande Berg 1984, Shum and McFarlane 1988), liver cirrhosis (Rungger-Brandle 1983), tumours (Schurch 1982), capsules around breast implants (Ryan 1974) as well as in normal wound healing.

Origin

The origin of these cells remains undecided. There is evidence to support the theory that these cells are modified fibroblasts, modified smooth muscle cells or a modification of some other cell type such as a macrophage (Reviewed by Ghadially 1982 and Skalli and Gabbiani 1988).

Modified Fibroblasts

In 1990, Darby, Skalli and Gabbiani used alpha-smooth muscle actin, smooth muscle myosin and desmin markers to label the cytoskeletal features of fibroblasts in healing rat wounds. They noted that, as wound healing progressed myofibroblasts expressed alpha-smooth muscle actin but not desmin or myosin (two markers strongly expressed in pericytes and/or smooth muscle cells). This lead them to

conclude that wound myofibroblasts were a partial smooth muscle differentiation of local fibroblasts. This confirmed other workers conclusions (Eddy et al 1988, Oda et al 1988, Vande Berg 1984, Hirschel 1971, Rudolph 1979, Ryan 1974, Madden 1974). The smooth muscle phenotypic change may have been induced by the action of Transforming Growth Factor beta-1 present in the wound environment (Roberts et al 1988).

Also, electron microscopic studies by Ghadially (1982) show that there are no myofibroblasts in normal human meniscal cartilage. However, when this structure is torn, myofibroblasts can be identified in the adjacent tissue. It is likely that these cells are derived from the local fibroblast population as the only other cell type present is the chondrocyte.

Modified Smooth Muscle Cells

Myofibroblasts in atheromatous plaques contain the alpha isoform of actin which occurs only in smooth muscle cells, and they contain desmin which does not occur in wound fibroblasts (Darby and Gabbiani 1990). It is therefore possible that these cells are derived from local smooth muscle cells whose rough endoplasmic reticulum has hypertrophied (Ghadially 1988). Indeed Ross (1968) noted that such a change can be produced in an oestrogen stimulated rat uterus.

Other Cell Type

Ryan et al (1973) found that intraperitoneal blood clots in rats do not become attached to adjacent surfaces but become covered by layers of myofibroblasts and mesothelial cells. The likelihood of macrophages transforming into

myofibroblasts was raised in this paper but this theory has not been substantiated by other authors (Ghadially 1982) until recently when Gown (1990) presented evidence that either of the above two cell types could have modified their phenotypes and acquired smooth muscle cell characteristics.

It therefore would seem reasonable to presume that cells with the electron microscopic characteristics of myofibroblasts can be derived from smooth muscle cells, fibroblasts or a variety of other cell types (Ghadially 1982, Gown 1990). The cell of origin probably depends on the predominant cell in the local cell population. In a recent review Gown (1990) suggested that the smooth muscle phenotype can be turned on in different environments. Assoian and colleagues (1984) have shown that the synergistic action of three cytokines from the alpha-granules of platelets can induce smooth muscle phenotypic transformation of fibroblasts. Darby et al (1990) found that smooth muscle phenotype induction can also be reversed, the timing of which, in the healing wound coincides with the cessation of wound contraction.

Immunofluorescent Evidence

In 1965 Johnson found that patients with autoimmune hepatitis produced an antibody to smooth muscle cells (Guber 1978).

Hirschel et al, in 1971, used human anti-smooth muscle serum to identify myofibroblasts in granulation tissue. Serum from a patient with chronic biliary cirrhosis was diluted 1:6 and applied to sections of various tissues. Sheep antihuman IgG fluorescent serum was used as a

counter staining reagent. Sections of rat stomach, caecum, kidney, urethra, bladder, all reacted with the anti-smooth muscle serum. The distribution coinciding with areas of smooth muscle cells stained, in similar sections, with haemotoxylin and eosin. This antibody also bound to the contractile fibroblasts in healing granulation tissue, normal fibroblasts were never stained. Human smooth muscle autoantibody was then shown to be an anti-actin antibody binding to the intracytoplasmic actin micro-filaments in smooth muscle cytoplasm (Gabbiani 1973). In 1986, Skalli and Gabbiani produced a monoclonal antibody against a synthetic decapeptide having the same NH2 terminal end as alpha-smooth muscle actin.

The use of a monoclonal antibody enables myofibroblasts to be clearly identified (McGrath 1982) by light microscopy. This means that larger biopsy specimens can be assayed so increasing the likelihood of the sample giving an accurate representation of the numbers of myofibroblasts in the wound (Guber 1978). Also, the considerable amount of time and experience needed to prepare the tissue for electron microscopy and to identify and count the myofibroblasts is not necessary when immunocytochemistry is used.

Activities

When strips of open wound granulation tissue (microscopically shown to be rich in myofibroblasts) are stimulated with substances that cause smooth muscle contraction (angiotensin, vasopressin, norepinephrine, bradykinin, epinephrine, and prostaglandin F-1-alpha) contraction occurs. These agents have a similar effect on avascular repair tissue which forms around intraperitoneal

haematomas in the rat, indicating that the vascular smooth muscle present in granulation tissue is not totally responsible for wound contraction (Ghadially 1982). Agents such as papaverine, and prostaglandins E1 and E2 (smooth muscle relaxants) provoke granulation tissue relaxation. Also, the distribution and numbers of myofibroblasts have been shown by some authors to correlate with the rate of open wound contraction (McGrath 1982, Baur 1978). This indicates that myofibroblasts, if not directly responsible for the contraction of granulation tissue in vitro and in vivo, are at least strongly associated with it.

Darby and Gabbiani (1990) further noted that during the phase of active wound contraction, alpha-smooth muscle actin was always present in the microfilament bundles of myofibroblasts.

When wound contraction diminished, the microfilament bundles did not stain with anti-alpha-sm-1 monoclonal antibody. This indicates that, as wound contraction slows down, actin is removed from the contractile fibres probably by the process of apoptosis (Darby 1990).

Rudolph (1979) has reported that similar cells are present in skin grafted wounds in rats and that their life span and numbers are influenced by the type of graft covering the wound. The wounds covered with full thickness skin grafts have fewer fibroblasts with a myofibroblast like appearance than split skin grafted wounds. These cells, also, are present for a longer period of time in wounds covered with split skin grafts.

Although this study lacked a truly objective assessment of myofibroblast numbers it did seem to show that

myofibroblasts were more numerous during the phase when the skin grafted wounds maximally contracted, a result similar to that of Guber (1978), McGrath (1982), Baur (1983) and Darby and Gabbiani (1990) who studied open wound contraction in various mammals. It would seem that during the phase of maximal wound contraction myofibroblasts are at their most numerous and they can be stained with anti-alpha-sm-1 monoclonal antibody.

Myofibroblast Numbers during Wound Contraction

If myofibroblasts are solely responsible for wound contraction then their numbers should correlate with the velocity of wound contraction. McGrath (1982) like Gabbiani (1973), Rudolph (1979) and Van de Berg (1984) believe that this is the case. Doillon et al (1987), looking at actin filaments in normal dermis and during various stages of healing of various different wound types did not find a direct correlation between wound contraction and the presence of actin staining fibroblasts.

However, the wounds made in this study did not include the panniculus carnosus, therefore this highly contractile structure was left behind and may have influenced the results. Other workers remove this structure as its presence results in confusion identifying the source of subsequent contraction. Doillon et al (1987) believed that actin containing fibroblasts were related to zones of trauma resulting from the skin grafting process used in their study. They report a total failure rate of 30% relating to the grafting process which perhaps indicates

that they were not familiar with this technique. This feeling is further substantiated when they report histological features of necrosis in several layers of the surviving skin grafts. This must cast doubt on their findings.

McGrath, Gabbiani, Rudolph and Van de Berg believe that animal open wound contraction begins after 4 days (when myofibroblasts begin to be identified) whereas Baur (1984) believes that wounds have already contracted by 25% by this time. Baur (1984) has suggested that in the early phase of wound contraction, the contraction force is generated by the circumferential edge of the surrounding skin. Specialized myoepithelial cells (Baur 1984) at the wound edge are strongly stained by antibodies to actin (Franke et al 1980) and are thought to be responsible for the initial wound contraction before 4 days. Peacock and Van Winkle (1970) and McGrath (1982) however, feel that all wounds initially increase in size following wounding and during the first 3-4 days the wound is in a preexponential phase of wound contraction. It only begins to contract after 4 days.

Most authors agree that after 4 days myofibroblasts can be seen in increasing numbers as wound contraction proceeds. In open wounds these numbers reach a maximum at 3-4 weeks. This corresponds to the phase of maximal wound contraction (McGrath 1982). When contraction stabilizes the myofibroblast numbers fall (McGrath 1982). In humans it has been demonstrated that myofibroblasts exist in hypertrophic scars (Baur 1975). They adhere to the surrounding structures by means of extracellular

fibronectin microfilaments (Baur 1983) and it is possible that these structures are disrupted when current splintage regimens are used to reduce scarring (Baur 1978).

Summary - Wound Contraction

Wound contraction occurs during the phase of fibroplasia (Clark 1988). Fibroblast migration and proliferation during this phase is probably influenced by the interaction between the many factors released from the cells present in the wound (Assoian 1988, McGrath 1990). It is probable these factors also influence the phenotypic metamorphosis of fibroblasts to myofibroblasts (Clark 1988).

Myofibroblasts are difficult cells to identify even with the help of monoclonal antibody reagents. They clump together and are sometimes associated with blood vessels (Baur et al 1978) making individual cell identification difficult. However they are present in many situations where active non-muscular contraction is taking place and have been identified by various authors in healing, contracting wounds. Recent studies (Darby et al 1990, Gown 1990) raise the possibility that actin is only present in the microfilaments of myofibroblasts when wounds are actively contracting.

Quantification therefore, of myofibroblast numbers with an anti-actin monoclonal antibody may give a numeric indication of the contractile nature of a healing wound.

1.3 Wound Dressings

History

The care of open wounds is probable as old as man himself. Skulls found by archaeologists have evidence of bone healing associated with either trauma or trephining indicating that the patient survived the procedure for quite some time (Knight 1985). The mortality associated with open wounds was probably very high but it may not have been as common as we might expect.

Two factors reducing mortality (Knight 1985) from trauma may have been;

1) the considerable infant mortality (two thirds of all births) which probably left the fittest for adulthood,

2) the sparsity of the population meant that cross infection with pathogenic organisms was less than today.

The aims of wound dressings in those days were probably to arrest bleeding, to cover the wound, and to attempt to splint any flail part. A multitude of agents was used to achieve these objectives.

The first documented medical and surgical techniques used for wound dressing dates from about 1500 B.C. as both the Ebers and the Edwin Smith papyri contain references to wound dressings. Up to the time of Christ the most advanced surgeons were probably the Hindus with Susrata being the most famous. Their favoured way of treatment was the application of bandages and poultices to a wound without further physically traumatising its surface.

In Europe, the Greeks dominated medical society even to the extent of forming the majority of Rome's medical fraternity. Hippocrates advised simple regimes in wound

healing, teaching that the wound edges should be kept dry and brought as closely together as possible to allow healing "by primary intention" which meant at that time without sepsis. Roman dressing procedures were based to some extent in science as they used metal salts as astringents and bound ulcers with figs which contain papain, an enzyme which dissolves slough.

Galen wrote of "laudable pus" in late Roman times and it would seem that these writings were misinterpreted following the fall of the Roman empire. Billroth in 1866 mentioned "laudable pus" and regarded it as beneficial. He noted that underneath it were healthy granulations which were essential for the subsequent healing process. Billroth felt these granulations were induced by the presence of pus. He felt that without the pus the granulation tissue would not appear so he assumed that pus was necessary for a wound to heal normally. The Church used its considerable influence to encourage this practice and many surgeons tried to incite sepsis in a wound believing that this was the natural way of healing (Knight 1985). Therefore wounds were traumatised with various mixtures, procedures and instruments to produce pus. Interestingly, it is documented that soldiers carried boxes of spiders webs with them into the Battle of Crecy, this believed to be the treatment for bleeding. Shakespeare also refers to the use of flax and egg white as treatment for a bleeding face wound in Midsummer night's dream and in King Lear. The scientific discoveries made by Pasteur and Lister in the 19th century changed peoples thoughts about wound healing.

The Twentieth Century

The discoveries of Louis Pasteur paved the way towards antiseptic surgery. This was further aided by Lister's carbolic spray. With the coming of aseptic surgery, dressings became sterile and the purpose of such dressings was to remove infected, necrotic debris and eliminate sepsis. Lumiere introduced "tulle gras", a type of paraffin impregnated gauze designed to reduce dressing adhesion, during the First World War and this type of dressing is still used today.

The aims of wound dressings since then have been primarily to remove infection and wound exudate, believing both to be linked. All substances in the wound environment were believed to be either detrimental or potentially detrimental to wound healing. The incorporation of antiseptics in the dressings emphasized that the destruction of infection (sometimes at the expense of the healing wound) was the prime concern.

Occlusive Dressings

For clarity the definition of an occlusive dressing is taken as

"a synthetic membrane that permits moist wound healing"

Eaglstein 1987

In 1958 it was observed that a blister healed quicker if it was left unbroken (Alvarez 1984). This finding stimulated research into the use of plastic films designed to mimic this natural situation. Winter (1962) noted that by covering a wound with an occlusive membrane and creating a moist wound environment there was almost a 50% increase in the speed of wound healing. Hinman et al

(1963) found that occlusive dressings had a similar effect on epidermal repair in incised wounds made on human skin. The fear that these dressings, by creating a pool of wound exudate, would increase the risk of infection has meant that they were slow to be used generally. Studies have shown that when these dressings are used judiciously the infection rate is lower than treatment with conventional dressings
(Mertz P.M. et al 1984, 1985).

Occlusive Dressings and Wound Contraction

Alvarez (1983) found that when occlusive dressings were used to dress superficial wounds, epithelialisation and underlying collagen synthesis were increased.
These findings indicated that a wound dressing can influence the healing process in those parts of the wound not in direct contact with the dressing.
Frank, in 1985, observed that an occlusive dressing (Biobrane) had the same effect in reducing wound contraction as a full thickness skin graft. This finding was endorsed by Forseman (1986). Both authors stated that, in order for this particular dressing to reduce contraction it must adhere to the underlying wound. Biobrane adheres to the wound in a manner similar to that of a skin graft (Frank 1984). It is possible that the outer layer of the dressing reduces water loss and the inner layer mimics dermal collagen. If these two conditions are met then early wound contraction (in rats) is reduced.
Winter (1972) showed that connective tissue regeneration began 3 days earlier when a wound was synthetically dressed. Linsky (1981) found that when synthetically

dressed wounds were compared with air exposed wounds there was a reduced number of inflammatory cells, fibroblasts and a reduced breaking strength. Dyson (1988) found that not only did synthetic dressings reduce the numbers of neutrophils and macrophages in a wound they also accelerated the inflammatory phase of wound healing. This resulted in the wound entering the collagen remodeling phase at an earlier stage.

Alvarez et al (1983) established that, when superficial wounds were covered with an occlusive dressing, their effect on wound healing was not influenced by the oxygen concentration at the wound surface. However, some studies (Silver 1972, Winter 1978) report increased wound oxygen tensions when occlusive dressings were used so the role of gaseous transfer by these dressings in wound healing is yet to be fully evaluated.

Eaglstein et al (1988) noted that there was a critical period in the wound healing process when the application of an occlusive dressing would produce optimal benefits in wound reepithelialisation. This period (between 2 and 24 hours after wounding) may be important in determining the subsequent course the wound follows.

It is possible that;

a) the composition of the fluid that accumulates under an occlusive dressing may be influenced by the dressing if it is applied early enough (Eaglstein 1984, 1988)
b) this altered fluid further influences the healing process in the wound.

Madden et al (1989) showed that when wound fluid taken from under an occlusive dressing was applied to a

keratinocyte culture there was an increase in cell proliferation. This increase was most marked when exudate from a burn wound was used. Other workers have noted an increase in cultured fibroblast cell division and growth when fluid from an occlusively dressed human full thickness wound was added to the culture medium (Alper 1985).

In summary it would seem that these dressings may reduce the early acute inflammatory response (Linsky 1981), accelerate the wound repair process (Dyson 1988) and may do so by localizing beneficial cytokines to the area of the wound (Brown 1986, Jonkman 1989,1990) so having a profound influence on the wound's subsequent behaviour. Other postulated reasons for the beneficial effects of occlusive dressings include:

the fluid (Eaglstein 1987), or semi-fluid (Jonkman 1990) environment created increasing cell migration (Madden 1989) and proliferation (Alper 1985),

the beneficial effect on the bacterial microenvironment (Spira and Hall 1973, Buchan et al 1981, Holland et al 1984, Mertz 1984),

and the maintenance of the electrical potential between the wounded and intact skin (Alvarez 1983).

Clinically, these dressings have been shown to be beneficial to wound healing (Spira 1973, James J.H.1975, Chvapil 1982, Ramirez 1984, Moserova 1987, Phillips 1989, Yang 1989). In a long term human study Hien (1988) found that full thickness wounds covered with an occlusive dressing healed quicker and left scars that were cosmetically superior to the conventionally dressed

control wounds. However, microorganisms can multiply and survive beneath occlusive dressings (Buchan 1981, Mertz 1984). Although one dressing (Opsite) has been shown to activate complement C3 giving it some bacteriocidal as well as leucocyte chemotactic properties (Holland et al 1984), care must be taken to choose the wounds which will benefit most from occlusive dressings.

The initial belief that these dressings would be a permanent substitute for skin has not been substantiated (Spira and Hall 1973) but their effects when used in conjunction with a skin graft have not been fully evaluated.

1.4 Skin Grafts

A skin graft is a portion of epidermis and dermis which has been completely severed from its original site and transferred to some other part of the body. Subsequent graft survival depends on the vascularity of the recipient area.

Skin grafts can be classified as;

Autografts...a graft coming from another area of the same individual

Allografts...a graft coming from genetically dissimilar individuals of the same species

Xenograft....a graft coming from a member of another species

Skin grafts can also be classified by their physical characteristics.

Full-thickness skin grafts

These grafts contain all the epidermis and all the dermis. Full-thickness skin grafts, by definition, contain all the dermal structures which provide a source of epithelial cells.

This means that;

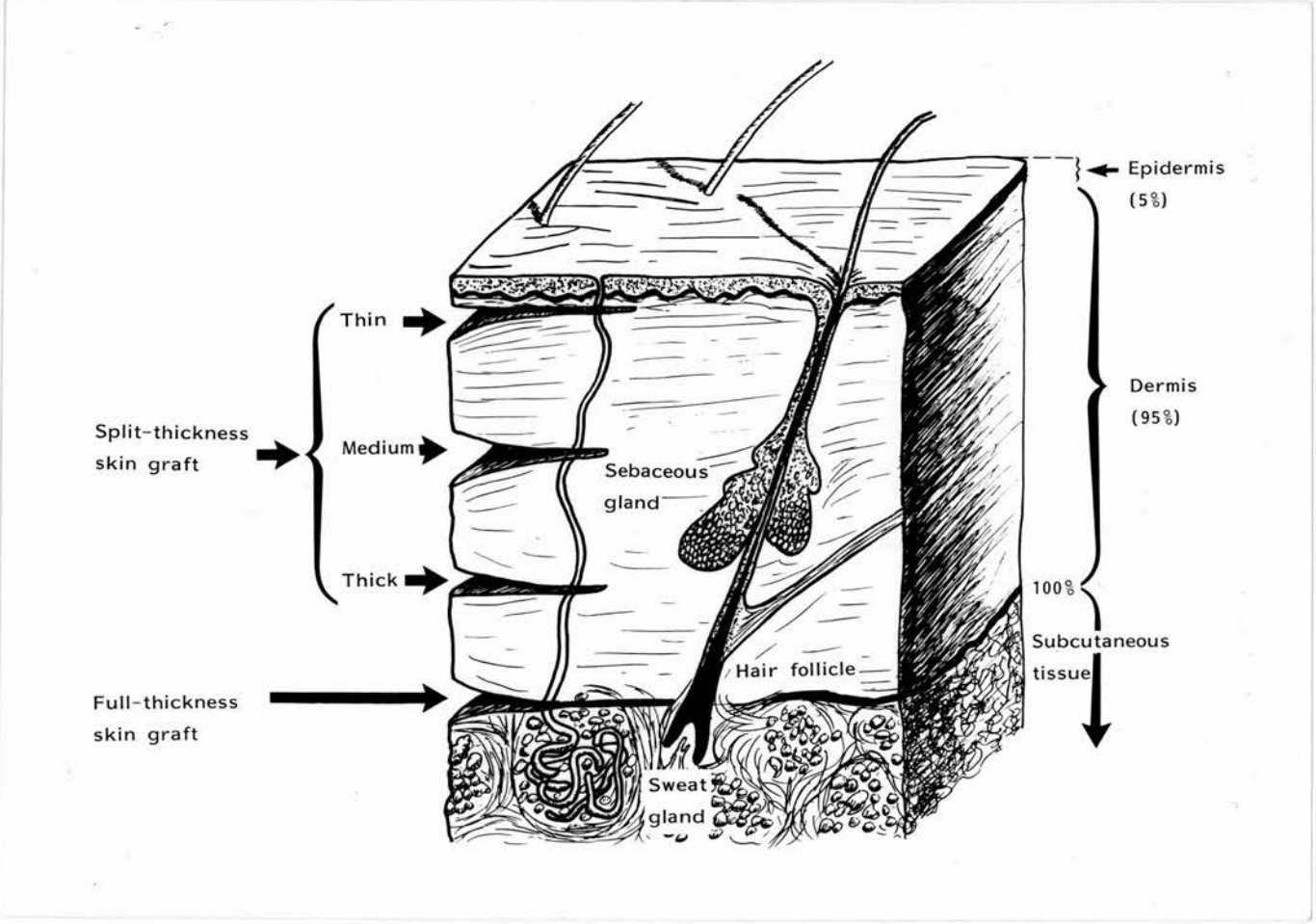
a) the graft, once transferred, will not contract and will exhibit the characteristics of normal skin (texture, colour, and secretions)

b) the donor defect left when the graft is transferred will heal by secondary intention unless otherwise treated. This factor usually determines the size of the graft (Grabb and Smith 1979).

Split-skin grafts

These grafts contain all the epidermis and a varying portion of the dermis. Split-skin grafts leave a portion of the dermis behind after transfer and it is from structures within this remnant that epithelialisation takes place, usually within 14 days. Further skin grafts can then be harvested from this area.

Split skin grafts can be subdivided depending on the proportion of dermis in the graft into thin, intermediate and thick grafts. The thicker the graft the less the underlying wound contracts but the resulting donor site takes longer to re-epithelialise.



History of Skin Grafts

In India, as early as 2500 B.C., Susrata treated defects with the use of composite free fat and skin grafts (Chick 1988). The skin of the buttock was marked (around a leaf cut to a pattern of the defect) and was then slapped with a wooden paddle until it became quite congested. The skin and underlying fat was then excised and transplanted onto the defect, its edges sewn to the freshened edges of the wound.

These techniques, carried out by the Brahmin Koomes Caste, (who made tiles and bricks and considered the repair of skin defects their province) were lost to time (Chick 1988).

In 1804 Guiseppe Baronio transplanted skin grafts in sheep and in 1817 Leroux, in Paris, performed skin graft transplantation according to the ancient Hindu techniques. Brock (1952) described how Sir Astley Cooper performed a human skin autograft in 1817. He cut a piece of skin from an amputated thumb and applied it to the amputation stump. In 1818 Bunger in Marburg (Germany) performed a skin grafting operation in the presence of three visiting doctors, one of which recorded details of the operation and the subsequent clinical events (Deelman 1925, Rudolph 1973). Full thickness grafts were successfully transplanted from the arm to the face by American surgeons in the early 1840's but little notice was taken of this breakthrough in Europe (Chick 1988). Jacques-Louis Reverdin, an assistant in the Hospital Necker in Paris, presented his technique of transplanting

skin islands to aid the healing of ulcers in 1869 (Rudolph 1973). It was not until some time later that Reverdin's technique became known to the medical establishment outside France as Paris had become isolated due to the Franco-Prussian War (1870-1871).

These techniques were taken up in London by Pollock with great success (Editorial B.M.J.1870) before being accepted in France because Le Fort and Blot had publicized their fears that the secondary defects caused by harvesting skin grafts may become portals for other disease processes (Chick 1988).

Reverdin's grafts accelerated wound healing but only partially reduced wound contraction. In 1872 Reverdin published his last work on skin grafting and in it he suggested that the technique could be used to improve the management of large burns (which usually took at least six months to heal) by speeding up epithelialisation and reducing wound contraction. In an attempt to reduce wound contraction even further, Ollier in 1871 used strips of epidermis harvested with a sharp knife and laid together to totally cover the wound. This method produced less contraction and scar formation, resulting in a better quality of skin. Thiersch in 1886 reported his method of harvesting strips of epidermis with a little dermis and applying these strips to a wound whose granulation tissue had been shaved with a sharp razor. He suggested that this method improved ultimate healing.

One year earlier Wolfe, an ophthalmologist in Glasgow, wrote of the use of a full thickness skin graft in the successful treatment of ectropion. His name became

synonymous with this type of graft.
In the last two decades of the nineteenth century Von Esmarch and Krause popularized the use of the full thickness skin graft and showed that it could be used to reconstruct a variety of defects.
It was not until the 1920's that the techniques and equipment were introduced to allow the reliable and accurate harvesting of split skin grafts. Finochietto designed a knife which could control graft thickness and 10 years later Humby produced his knife, a modification of which, is used in many plastic surgery units today.

Healing Processes in Skin Grafts

When a skin graft is harvested all connections between it and the donor site are severed. It's survival depends on the establishment of a stable attachment by an elastin-fibrin bond (Burleson 1972) to the recipient area which will then allow neo-vascularisation to occur.
During the first 24-48 hours the graft survives without a circulation (Rudolph 1973). Fluid from vessels in the underlying wound (Kikuchi 1970) moves in and out of the vascular channels in the graft (Converse 1957), but probably provides no nutritional role (Clemmesen 1962).
Clemmesen suggests that the graft is held to the underlying wound by a fibrin meshwork. This mesh allows fluid to pass to and from the graft in the first 24-48 hours. The fluid keeps the graft moist and prevents the vascular channels in the graft from closing. Thereafter the interstices of the mesh become lined with vascular endothelium from the underlying wound which then links

with the vascular channels in the graft (Rudolph 1973). Haller and Billingham (1967) believed that the original graft vessels were reused but evidence from Zarem (1967) suggests the intrinsic graft vessels merely act as non-living conduits. This work has been substantiated by enzymatic studies carried out by Converse (1962) and Wolff (1965).

As the wound matures the fibrin-collagen bond which originally held the graft to the wound is degraded. Fibrin degradation products, acting competitively with thrombin, reduce the further deposition of fibrin so acting as a negative feedback system. The collagen on the dermal side of the graft now is intimately associated with the newly forming fibrotic part of the wound. The graft is now (after 6-10 days) firmly attached to the wound. Clinically, it is deemed to be healed and has a pink appearance indicative of the amount of patent blood vessels in the graft. In humans the graft wound interface continues to remain active for many months, this manifests as graft, or wound contraction (Perkins 1987). Studies in rats have shown massive turnover of collagen in skin grafts with significant differences between full thickness and split skin grafts (Klein 1974).

Contraction of Skin Grafts - Definition

Primary Contraction

When a skin graft is harvested it undergoes immediate shrinkage (Davis and Kitlowski 1932). This is often termed primary contraction (Grabb and Smith 1979) and is probably due to the unopposed recoil of the dermal

elastic fibres (Rudolph 1973). It can be reversed when the initial tension on the graft is re-established.

Secondary Contraction

This is synonymous with the term "underlying wound contraction" as it is the underlying wound that contracts, not the skin graft. (Brown and McDowell 1958) The amount and rate of contraction is influenced by the type of graft covering the wound, full thickness skin grafts reducing wound contraction more than split skin grafts of similar thickness (Corps 1969).

Skin Grafts and Wound Contraction

Padgett in 1942 noted that thin split skin grafts "took" very well but the underlying wound contracted by as much as 60% especially over the flexor aspect of a joint. When thicker skin grafts were used the resultant wound contraction was much less (30%). The concept then, was that the thicker the split skin graft the less the underlying wound would contract. The Padgett dermatome was developed to harvest as thick a split skin graft as possible to reduce the subsequent recipient area contraction and still leave a self healing donor site.

In 1969, Corps stated that there was a great variation of contraction at various times during the healing process. Studies by Ragnell (1952), Corps (1969) and Sawhney (1971,1977) all showed that all skin grafted wounds contracted in the first 3-4 weeks following application. Wounds covered with full thickness skin grafts contracted less than those covered with split skin grafts. After 4 weeks all wounds increased in size, the ones covered with

a full thickness skin graft enlarging more than those covered with a split skin graft. In Corps's study (1969) full thickness skin grafts did not immediately stop wound contraction. In the first 4 weeks wounds covered with a full thickness skin graft contracted to 65-80% their original size, split skin grafted wounds contracting to 25-45% their original size during the same time. The time of maximal contraction in animal skin grafted wounds coincides with the early phases of wound healing characterized by inflammation and collagen deposition (Pessa et al 1987).

The phase of collagen remodeling is the phase when the contrast between wounds covered with different skin grafts manifests most markedly. The full thickness skin grafted wounds increasing to about twice the size of those wounds covered with split skin grafts (Corps 1969). This phase coincides with the phase of scar widening in animals indicating that during this time the collagen remodeling is associated with a reduction in the force of wound contraction so allowing the wounds to increase in size (Corps 1969).

Skin grafted burn wounds go through similar phases of wound healing before a stable result occurs (Baur, Parks and Larson 1977). The time taken for the wound to go through the various phases of wound healing is however longer in the human. The phase of scar formation is associated with an inflamed appearance of the tissues and may last from 3 to 6 months in a non-burn wound (Ketchum 1979) and from 3 months to 3 years in a burn wound

(Larson et al 1974, Baur, Parks and Larson 1977).
The above studies indicate that the dermal component of a skin graft has some function in influencing underlying wound contraction, the thinner the skin graft the more the underlying wound will contract (Blair and Brown 1929). How it does this is unclear.

1.5 Present Clinical Situation

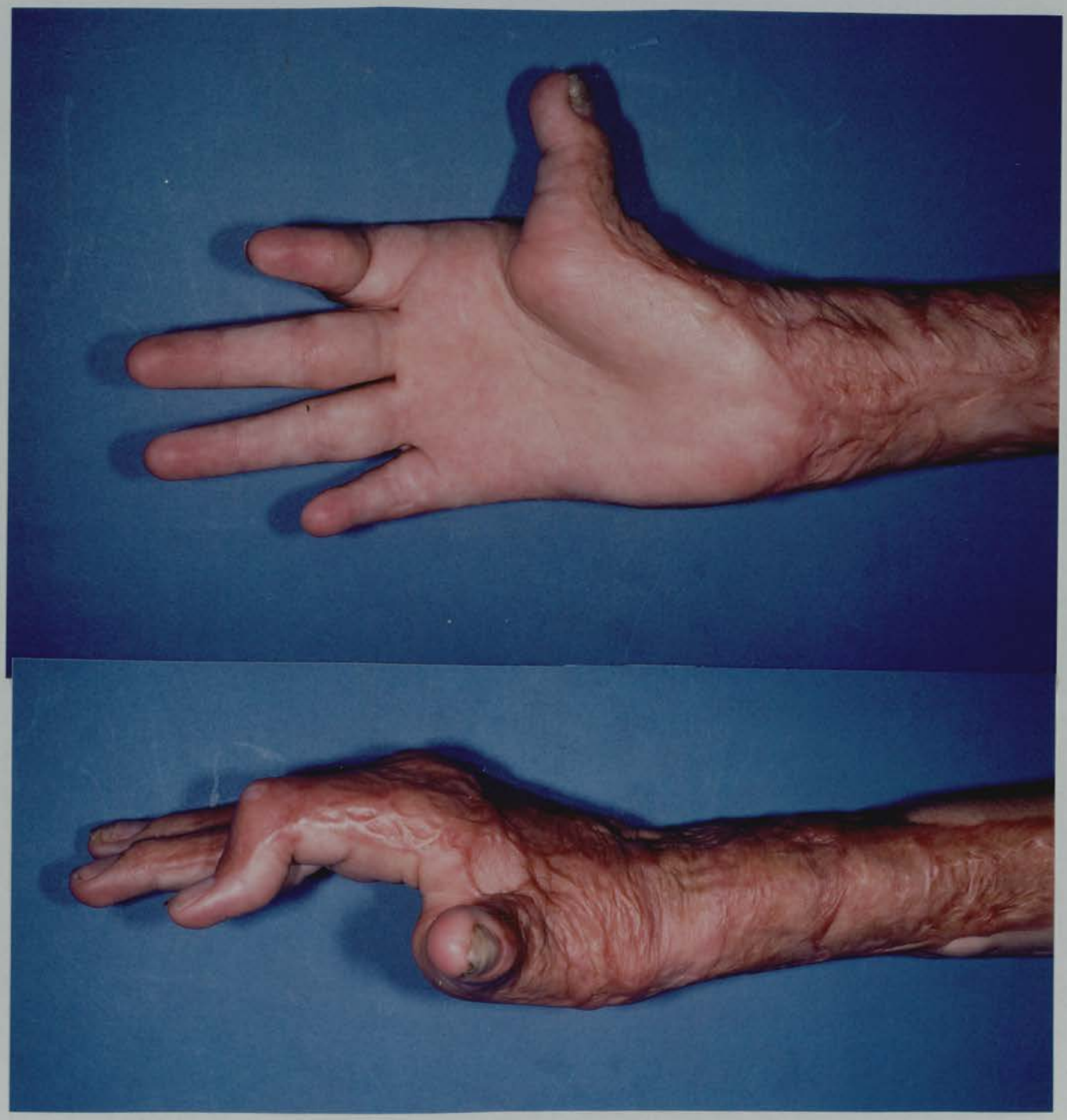
Large, open wounds are routinely produced in surgery as a result of trauma, tumour excision and most commonly following a burn involving the full thickness of the skin. Covering these wounds with a skin graft allows rapid epithelialisation to take place which provides a barrier to infection and reduces pain.
In the clinical situation a thin split skin graft will revascularise more readily (Grabb and Smith 1979) and leave a donor site that heals quicker than a thick split skin graft. Thin split skin grafts also survive with greater certainty in contaminated wounds than thicker split skin grafts (Peacock and Van Winkle 1970).
The main objective in the early part of wound healing is to achieve rapid epithelialisation and to concentrate on the reduction of wound contraction at a later stage. Unfortunately, the present regimes are not 100% successful in reducing wound contraction (Perkins 1987) and the resultant scarring can be cosmetically (Fig.1) or functionally (Fig.2) debilitating (Baur, Parks and Larson 1977) .

Fig.1

This picture illustrates the cosmetic deformities resulting from the contraction of wounds produced when full thickness burns of the knees, legs and ankles are excised and covered with split skin grafts.

Fig.2

The contraction of the split skin grafted wounds over the dorsum of the fingers, thumb and wrist has resulted in a fixed deformity of these areas which has considerably reduced the functional capacity of the hand.



Burns

Split skin grafting is commonly carried out in the treatment of burns. The timing of the grafting procedure is influenced by the systemic effect of the burn and the likely systemic effects of sometimes quite extensive surgery.

The following gives an indication of how skin grafting fits into the general management of the thermally injured patient.

By convention burn management can be broken down into several phases.

Initial Care

The aim in this part of the treatment programme is to stabilize the patient from the systemic effects of the burn therefore reducing the risks of subsequent surgery. Burn injured patients are usually assessed in non-specialist hospitals. Those requiring specialist care due to the depth and extent of their burns are transferred to a regional burns unit. On arrival at the burns unit specialized investigations are carried out and treatment is commenced.

The objectives are:

a) to assess the amount of fluid needed to replace that lost as a result of the burn injury and
b) to prevent or detect and treat the associated complications.

To this end various intravenous fluid regimens have been devised (Settle 1986), all depend on an assessment of the extent and depth of the burn. The amount given varies with the type of fluid used but the aim is to replace the

fluid lost from the intravascular space as a result of the burn injury. This fluid resuscitation takes place during the first 36-48 hours following injury.

Burn Dressings

A variety of burn dressings are used either with or without systemic antibiotics in an attempt to reduce wound infection and septicaemia (Jackson, Lowbery and Topley 1951, Peacock and Van Winkle 1970).

Surgery

Once the patient finishes the resuscitation phase of treatment, surgery to replace the burned skin is considered. Such surgery takes place either at an early stage, between 3 and 9 days post burn or at a late stage after 14 days post burn.

The aim of surgery at this stage is to provide a viable epithelial barrier to cover the burn wound. This reduces fluid loss and the likelihood of septicaemia (Baur, Parks and Larson 1977), as bacterial numbers in granulation tissue are reduced following skin grafting (Eade 1958, Zietkiewicz 1967).

Recent evidence (Jackson and Stone 1972, Lawrence and Stone 1973, Burke et al 1974, Engrave et al 1983, Scott-Conner 1990) suggests that skin grafting results in rapid wound closure and minimizes tissue loss. This leads to a reduction in the risks of septicaemia, reduced morbidity, reduced nitrogen loss, a shorter hospital stay, a reduction of wound contraction and subsequent hypertrophic scarring in full thickness burn wounds. Split skin autografts also promote the maturation of collagen fibres in burn wounds (Takayanigi et al 1989),

and, if grafting is carried out at an early stage, mortality may be reduced (Hendon et al 1989).

The harvesting of split skin grafts has been made easier with the introduction of powered dermatomes that can be set to harvest grafts of specific thickness.
Different techniques of graft preparation including meshing (Tanner, Vandeput and Olley 1964, MacMillan 1970) and perforating help reduce the incidence of post operative failures resulting from haematoma and seroma formation.
Skin grafts can be stored and applied (within 21 days) when the wound conditions permit so increasing graft survival. (James and McGrouther 1985)

Wound Contraction

In burns surgery split skin autografts are used routinely to cover the excised burned area. Although split skin grafts do reduce wound contraction (Takayanigi et al 1989) the pathophysiology of a burn wound results in excessive apparently disorganised collagen formation which often manifests in the formation of hypertrophic scars and contractures (Larson et al 1974).
Usually 2 to 3 months after the burn wound has healed it becomes elevated and hard. It is characterized by a marked increase in vascularity, fibroblasts, myofibroblasts, interstitial material and oedema (Larson et al 1979). This phase of healing can last from 3 months to 3 years and if the associated wound contraction is not treated scar and joint contractures result (Larson et al 1974).

Myofibroblasts are first noted in burn wounds 3-5 days after trauma. They usually contain large amounts of rough endoplasmic reticulum indicating that they are actively engaged in protein synthesis (Baur, Parks and Larson 1977). As wound healing progresses myofibroblasts become more numerous but their rough endoplasmic reticulum content falls. Indeed, at the peak of the contraction phase, almost all scar fibroblasts have a myofibroblast appearance (Baur et al 1978).
The role of these cells at this stage is likely to be contractile rather than secretory. Myofibroblast contraction results in a bending of the collagen fibres which may produce the microscopic sinusoids seen in hypertrophic scars (Baur, Parks and Larson 1977).

Attention is focussed on reducing wound contraction following surgery when the skin grafts are stable (Larson et al 1974). Regimens are implemented incorporating physiotherapy, pressure garment use (Harris and Pegg 1989), splintage (Cronin 1961, Willis 1970) and possibly secondary surgery (Evans, Larson and Yates 1968, Dobbs and Curreri 1972). These regimens often continue for many months or years after the initial skin graft operation (Larson et al 1974).
This is consuming in terms of time, for the patient and the staff, and money in terms of lost patient earnings and resources allocated to these regimens (Perkins 1987). In the Regional Plastic Surgery Unit at St.Andrew's Hospital, Billericay, Essex the annual budget for Paramedical staff and resources allocated to treating the

effects of wound contraction is £80-90,000. A similar sum is spent in the Welsh Regional Burns and Plastic Surgery Centre in Chepstow.

These regimens are considered to be successful if flat, supple, stable scars are produced. When this is achieved many scars are still cosmetically unattractive and functionally disabling (Baur, Parks and Larson 1977).

Chapter Summary

Wound contraction has beneficial effects but it often leads to disappointing results following skin grafting surgery for burn injuries.

Many post operative regimens exist to attempt to reduce this phenomenon but this late wound manipulation often produces poor cosmetic and functional results. A regime involving early wound manipulation therefore may have better results.

Wound Contraction

The definition of wound contraction by Van Winkle (1967) as "the diminution in size of an open wound which is the result of the centripetal movement of the whole thickness of the surrounding skin" indicates that it has been assumed that wound contraction occurs only in open, full thickness wounds. Literature concerned with wound contraction commonly refers to open wounds with attention being given to the role of granulation tissue in this process (Skalli and Gabbiani 1988). However contraction can occur in wounds devoid of granulation tissue such as skin grafted wounds (eg.Corps 1969) and incisional wounds

(Peacock and Van Winkle 1970, Ketchum 1979) indicating that this phenomenon may be an integral part of the normal healing process of all wounds (Frank 1985). Factors affecting the complicated process of wound contraction are probably present from the very initiation of the wound healing process (Tsuboi 1990). The initial cellular influx releases factors which stimulate the migration, proliferation and the likely phenotypic transformation of fibroblasts (Clark 1988). Collagen formation and wound contraction are probably so fundamentally linked that it is not unusual therefore to find that wounds contract maximally during the fibroplastic phase of wound healing (Clark 1988). The time this phase occupies varies with the type of wound and the animal species studied. It lasts approximately 3-4 weeks in the rat and up to at least 6 months in humans (Grabb 1979). Its cessation coincides with the commencement of the final phase of wound healing where collagen metabolism stabilizes and the wound (or scar) enlarges (Corps 1969).

Factors affecting Wound Contraction

An argument could be proposed stating that factors influencing wound contraction are:

a) released as a result the creation of the wound and
b) influenced by the environment of the newly formed wound.

Factors such as wound depth and subsequent dessication may be influential in establishing this environment.

Wound Depth

The anatomy of the cutaneous tissue remaining following wounding may influence the subsequent physiological responses to the wound environment.

Epidermal Loss

Epidermal stripping experiments indicate that epidermal loss is replaced by regeneration of cells mainly from the basal layer adjacent to the dermal-epidermal junction. Healing by contraction is not reported to take place (Peacock and Van Winkle 1970)

Dermal Loss

When the superficial layers of the dermis are removed, for example in the harvesting of a split skin graft, the resultant wound heals mainly by re-epithelialisation from the remnants of the dermal adnexal glands. It would seem logical to assume that the role wound contraction plays in wound healing becomes more apparent as more of the dermis is removed.

When the number of epidermal remnants (eg. sweat glands and hair follicles) falls below a certain figure wound healing mainly by re-epithelialisation is no longer possible and healing by wound contraction occurs. The above occurs when burns penetrate to the deeper aspect of the dermis.

When no dermal epithelial remnants exist ie a full thickness defect, wound contraction is maximal.

Therefore, it would seem that wound contraction is inversely related to the amount of dermis left in the wound. The greater the percentage of intact dermis left the less the wound will contract.

Tissue Replacement

When the tissue lost during the creation of a full thickness defect is replaced, subsequent wound contraction is reduced. This reduction is again related to the amount of dermis present in the replacement tissue (skin graft). Grafts containing only the epidermal aspect of the skin (Padgett 1942) do not affect wound contraction. When thin split skin grafts (with a small amount of dermis) are used, wound contraction is minimally reduced. The thicker the split skin graft used, the more it reduces underlying wound contraction.

Full thickness skin grafts (with the full complement of dermis in situ) are the most effective grafts in reducing wound contraction. Wounds covered with full thickness skin grafts however, still contract and in rats this can be up to 40% during the first 4 weeks after wounding (Corps 1969). The full thickness skin grafted wounds then enlarge during the remodeling phase of wound healing. Wound contraction therefore still persists even when all the elements of the dermis removed during wounding are replaced. When all the elements of the skin (epidermis, dermis and subcutaneous fat) are surgically replaced and accurately approximated (the situation in an incisional wound) (Gillman, Penn et al 1952, Grabb and Smith 1979), wound contraction still manifests.

Three to four weeks after wounding, a normally healing human scar is hyperaemic and ridged (the major clinical sign of active scar formation - Baur et al 1977) and has approximately 30-40% the strength of normal skin (Ketchum 1979). The linear scar will then undergo contraction

(Peacock and Van Winkle 1970) and over the following 3-4 months the hyperaemia will fade and the scar will widen as the collagen remodels. Although the contraction of incisional wounds has not been accurately quantified it is known from clinical practice that it manifests during the first 6 months following surgery - the time span of the fibroplasia phase (Ketchum 1979).

If all the elements of the skin are replaced the stimulus to contraction still exists and continues to manifest until the end of the fibroplastic phase of healing. To reduce this contraction even further, measures which influence wound healing at a more fundemental level may be required.

Burns

If a burn destroys the dermis which includes the sweat glands and hair follicles spontaneous reepithelialisation is no longer possible (Jackson and Stone 1972). The main aim of burn treatment is initially to preserve life and then to reduce the loss of function associated with post treatment scarring. The problem of infection in the burn wound requiring skin grafts is reduced either by removing the necrotic tissue surgically (Jackson and Stone 1972), or conservatively, with regular dressing changes (Wallace 1952, Lynch 1979). Split skin grafts are then applied to speed up epethelialisation and reduce scarring (Takayanigi, Hatano and Aoyama 1989). They also reduce the bacterial count of the contaminated wounds (Eade 1958, Allen et al 1973) so reducing the risk of systemic sepsis (Takayanigi, Hatano and Aoyama 1989).

Thin split skin grafts are used for 2 reasons.

Firstly their donor sites heal quickly so reducing that portal to infection and allowing further harvesting to be carried out (Peacock and Van Winkle 1970).
Secondly thin split skin grafts are associated with a higher percentage of complete take in the presence of excessive exudation or moderate infection (Allen et al 1973).

"Nearly every severe burn wound results in the formation of a scar" -Baur, Parks and Larson (1977), so once complete epithelialisation has occurred and the skin grafts are stable, attention is turned to the reduction of the contractures induced by the contraction of the skin grafted wounds (Peacock and Van Winkle 1970).
This wound contraction occurs in the phase following successful skin grafting and may last up to 3 years (Larson et al 1974). The skin grafted wounds have an inflamed appearance and a tremendous amount of collagen is deposited in the form of mats laid down in omidirectional arrays (Baur, Parks and Larson 1977).
It would seem therefore that the burn wound remains in the inflammatory phase of wound healing for an excessive length of time. This is probably because damaged, viable tissue is (purposely) left when burns are surgically excised (Jackson and Stone 1972) or the deeper aspect of granulation tissue is skin grafted when burns are managed conservatively (Grabb and Smith 1979).
These tissues may continue to stimulate the inflammatory response and one manifestation of this is the excessive wound contraction and scarring associated with these

injuries.

Wounds covered with thin split skin grafts contract more than those covered with thick split skin grafts or full thickness skin grafts (Padgett 1942, Corps 1969). They do so by contracting to a greater degree during the fibroplastic phase of healing and subsequently expanding to a lesser extent during the collagen remodeling phase (Corps 1969).

The conventional methods or reducing such scarring is the application of splints, pressure garments, the use of physiotherapy and occupational therapy with the prospect of secondary surgery if these regimens are not successful. Even if these regimens are successful the cosmetic results are often less than satisfactory (Baur, Parks and Larson 1977).

Could the early manipulation of the wound environment reduce this contraction?

Wound Dressings

The main principles of wound dressings until the late 1950's were solely concerned with the eradication of infection (Wallace 1952). Winter (1962) noted that when a wound was allowed to heal in a moist environment wound healing was increased. Dyson (1988) found that this effect manifested even in full thickness wounds. She noted that the wounds spent less time in the inflammatory phase of wound healing when they were covered with an occlusive dressing (Opsite - Smith and Nephew U.K.Ltd.). If an occlusive dressing reduced the time and intensity of the inflammatory phase of wound healing could it reduce wound contraction? Initial reports (Frank

1984,1985, Foresman 1986) suggest that the application of synthetic occlusive dressings can reduce initial wound contraction when used to cover open wounds in animals. A subjective review of burns treated with synthetic dressings indicated that these dressings could reduce hypertrophic scarring associated with deep dermal injuries (Grisolia et al 1991).

The exact manner in which they do so remains unknown although several theories have been proposed (see earlier section).

The occlusive dressing used in the above studies (Biobrane) consisted of an ultrathin semipermeable membrane bonded to a nylon fabric. This is coated with polypeptides from porcine dermal collagen. It adheres to a wound in a manner similar to that of a split skin graft (Frank 1984). This adherence is temporary in nature lasting for only a few weeks.

The two fractions of Biobrane could be mimicked by more permanent substitutes. The porcine dermal collagen could be replaced with a split skin graft. This would provide a source of collagen which was covered with a viable host epithelial layer which would be permanent in nature. The occlusive properties of the nylon/silicone outer bilayer of Biobrane could be copied with another synthetic dressing which would adhere to the surface of the skin graft (Opsite). If this newly created "permanent dressing" has an effect similar to Biobrane on wound contraction then it may reduce the contraction of wounds covered with split skin grafts to that of those covered with full thickness skin grafts.

CHAPTER TWO

A Study of the Effect of an Occlusive Dressing on Human Wound Contraction

2.1 Experimental Design

Aims

This study was set up to see if synthetic dressings altered human open wound contraction in the same way as they altered wound contraction in rats.

Materials and Methods

Split skin graft donor sites are made in a repeatable controlled procedure, can be treated in a similar manner, and healing is independent of the primary defect for which the skin graft is being used.

The dressing used in this study (Transigen, Smith and Nephew Ltd.) is a synthetic wound dressing designed to allow excess moisture to evaporate in a controlled manner ensuring the wound is always kept moist.

Fifty five donor sites on fifty five patients were studied. All donor sites were on the thigh as large donor sites (at least 10x5 cm)were used to minimize experimental error. Patients with more than one donor site per thigh were excluded so that all wounds studied were surrounded by normal skin.

Donor site size was assessed in theatre by compressing a sterile paper towel to the area after allowing capillary bleeding to take place. The mark made was carefully cut out and re-applied to ensure accuracy. An acetate sheet tracing was later taken and the initial towel discarded. The dressing regimen was then chosen by a randomizing technique allocating the donor site to be dressed conventionally or with Transigen.

Conventional dressing consisted of covering the wound with paraffin impregnated gauze then dressing swabs covered by cotton wool held in place with a crepe bandage.
Transigen dressing regimen consisted of drying the surrounding skin with a gauze swab and then applying the dressing and loosely covering this with a crepe bandage.
Both dressings were left undisturbed for two weeks. The dressings were taken down in either the outpatient or ward environment. Tracings were taken of the epithelialised areas on acetate sheets and the surface areas calculated and compared.
Refer to Appendix 4 for details.

2.2 Results

Failures

Infection occurred in two donor sites covered with Transigen and three conventionally dressed donor sites. These were excluded from the study and responded to local dressings treatment.

The composition of both groups was similar with regards to age and sex.

	NUMBER of PATIENTS	AGE RANGE	MEAN AGE
MALE (C)	10	39-61	49.5
FEMALE (C)	15	50-67	59
MALE (T)	14	43-64	53
FEMALE (T)	11	45-69	59.4

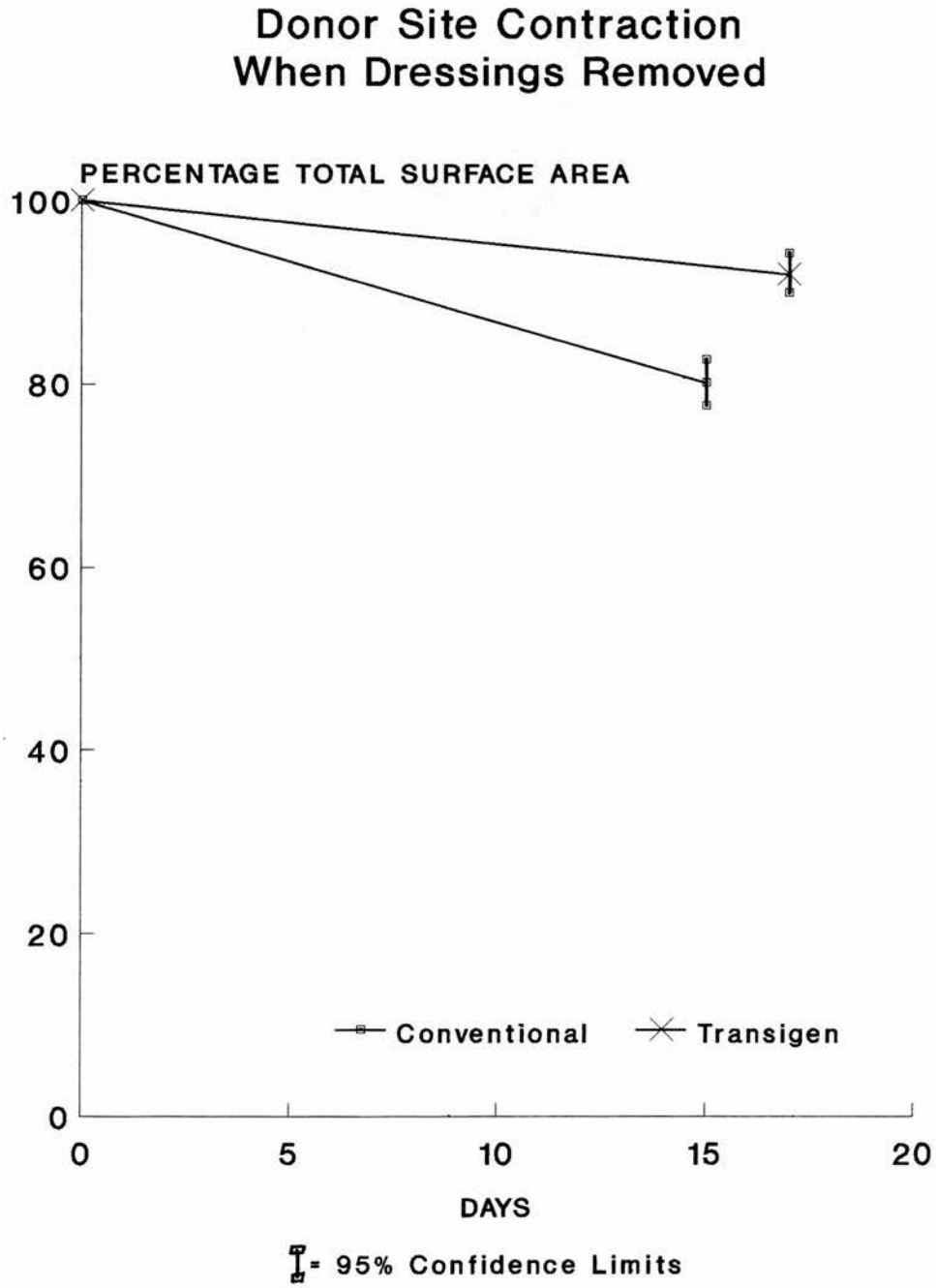
C = Conventional Dressing

T = Transigen Dressing

The mean time from graft harvesting to dressing removal was 15.3 days (standard error of the mean 1.9 days)in the conventionally dressed group and 17 days (standard error of the mean 1.5 days) in the Transigen group.

Mean wound contraction was 20% in the conventionally treated wound and 8% in the Transigen dressed group.

(See Appendix 4)



An unpaired "t" test comparing the differences in contraction in both groups at dressing removal shows $p < 0.001$

Conclusions

It is accepted that split skin graft donor sites are not full thickness wounds but this study showed that these wounds do, in fact, contract in the short term and this contraction can be altered by the wound dressing.

In order to minimize patient discomfort the trial was designed, with a professional statisticians advice, so that skin graft thickness would not be measured and the underlying dermis would not be biopsied. All wounds studied would be from the same anatomical area (thigh) and would be randomly allocated to each dressing group (unknown to the same surgeon harvesting all the grafts). This, coupled with the fact that there would be 25 donor sites in each group meant that both groups could be considered statistically comparable. Maximum wound contraction occurs in rats at 2-4 weeks. This coincided with the clinical practice of removing donor site dressings so measurements were taken at that time.

It would seem that partial thickness wounds, in humans, do contract. This contraction can be measured as early as 14-17 days after wounding and can be influenced in humans as it is in rats if the wound is dressed with a synthetic dressing.

Summary

Since humans respond in a similar manner to rats when open wounds are synthetically dressed then the results of a study in rats on the effect a synthetic dressing has on the contraction of wounds covered with split skin grafts may be extrapolated into the human population with more confidence.

A Study of the Effect of Covering a Split Skin Graft with an Occlusive Dressing

Chapter Three

Animal Study

3.1 Experimental Design

3.2 General Layout

3.3 Animal Model

3.4 Skin Graft Harvesting

3.5 Moisture Vapour Transmission

3.6 Surface Area Measurements

3.7 Myofibroblast Estimation

3.1 Experimental Design

This work would be carried out in the rat as this animal has been used as a successful wound healing model by other workers investigating wound contraction (reviewed by Richey K.J. et al 1989).

General advantages of using the rat are:

a) many animals can be studied simultaneously at a low cost
b) the size of the animal means experiments could be carried out with one operator
c) individual housing of all the animals in a single room allows accurate standardization of each animals environmental temperature and humidity levels during the study.
d) Though the histology and morphology of rat skin is very different from that of human skin, rat wounds share the ability to contract, albeit in a more intense manner during normal healing. This exaggerated response is advantageous since this work proposes to study wound contraction rather than other aspects of wound healing (Frank 1985).

Particular advantages of the rat model are:

a) the effect of synthetic dressings on open wound contraction (Frank 1984, 1985, Foresman 1986),
b) the contraction of skin grafted wounds (Corps 1969) and
c) myofibroblast cell populations in healing wounds with (Rudolph 1979) and without (Gabbiani 1972) skin grafts have been studied in this animal.

Data from control wounds therefore, could be compared with

published work.

Experiment Objectives

The main aim of these experiments was to gather data which could be statistically analysed which would show the effect, if any, a synthetic dressing had on several factors of wound healing. All three experiments (Moisture Vapour Transmission, Surface Area Estimation and Myofibroblast Cell Population) would be carried out by one investigator on one population of rats. The aim was to compare the dressing's effect on all of the above parameters so a model was created which would allow in-animal control wounds to be studied. With a statistician's help a protocol was designed to allow the rapid repeatable formation of two wounds on a non-flexor aspect and two wounds on a flexor aspect of a rat. These wounds would be covered with a split skin graft fashioned from the tissue excised in the creation of the original wounds.

Animals would be grouped to allow:

A) the controlled application and removal of dressings at predetermined intervals,

B) measurements of moisture vapour transmission from and surface areas of the skin grafts at regular intervals throughout the study,

C) whole wound biopsies to be carried out. This would allow myofibroblast cell estimation to be undertaken without influencing the surface area experimental data.

Using 4 wounds per animal a total of 200 rats grouped into 25 groups of 8 would be studied. This would be the minimum number of animals which would allow the collection of data

that could be evaluated using paired statistical analysis, allowing for a 25% animal interference rate.

3.2 General Layout

The animal experiments were carried out in the Research Division of SMITH AND NEPHEW U.K. Ltd. This establishment has been designated by the Home Office as an approved place to carry out animal work.

I was allocated three rooms in this modern animal house. The temperature, humidity and light in these rooms could be individually controlled (see Appendix 1) thus standardising several important aspects of the study.

One room was used as an operating theatre so this room was used when the initial wounding and grafting procedure was carried out. It was also used when dressings were changed and measurements and biopsies were taken. The second room was used as a recovery area. This room was equipped with a separate heat source which was used to keep the animal's temperature stable. This post operative support resulted in a very low operative and post operative mortality rate.

The largest of the three rooms was used as the residential area. In this room all the rats were individually caged after the initial operation until the end of the study.

Pre-operatively rat weights were checked on a weekly basis and they were only entered into the study when rat weight approached 350 grammes. Immediately post skin grafting, as intra-muscular and intra-peritoneal drugs were used, the rats were checked on a half hourly basis usually for about three hours or until they had fully recovered from the

anaesthetic. Thereafter, the rats were checked three times a day to make sure the dressings were intact and weighed on a weekly basis (see Appendix 3.)
Following subsequent dressing changes and measurements (done under gaseous anaesthesia with a recovery time in seconds in this animal) the rat was monitored in the operating room and then returned to the residential room where routine checks were made.

3.3 Animal Model

Previous authors had used genetically similar rats with some rats being used as donor animals, taking no further part in the study (Millington 1968, Rudolph 1971, Smahel 1986). We decided to use a population of genetically dissimilar rats, all of which would take part in the experiments. The results of which, could be more plausibly extrapolated to the (genetically dissimilar) human population.
This meant that an animal model had to be designed which would allow,

a) the formation of bilateral wounds on a flexor (hip crease) and non-flexor (flank) surface and
b) the harvesting of split-skin grafts from the skin excised in the creation of these wounds. The benefit of this would be to reduce the overall assault on the animal.

The project licence issued under the home office regulations [ANIMALS(Scientific Procedures) Act 1986 Chapter 14 Section 5] permitted up to 5% of the animal's surface area to be used for this purpose. The animal model was therefore designed to allow a 2x2 cm. wound to be ex-

cised on the flank and hip flexor area of both sides of the rat.

Skin Graft Harvesting

The skin from the excised tissue would be used to harvest a split-skin graft which would be used to cover the newly fashioned open wound. This technique, specifically designed for this thesis, would utilize between 3 and 4 percent of the rat's surface area, depending on the weight of the individual animal. Skin graft thickness was measured to ensure similarly thick skin grafts were studied. (Appendix 2)

The skin grafts on one side of the rat would be covered with a synthetic dressing and the skin grafts on the other side would act as an in animal control.

Wound Biopsies

Studies have been published where surface area estimations have been carried out on skin grafts which have been regularity biopsied. It was felt that even the smallest biopsies would leave open wounds which would heal by secondary intention. This would affect the surface area of the skin graft so the study was designed to allow whole wound biopsies to be taken. This meant that whole groups of rats were sacrificed at regular intervals throughout the study to provide biopsy data which could be analysed statistically, still leaving enough animals in the study to allow the other observations to be carried out throughout the 15 week period.

Animal Groups

Individual groups of eight rats per group were studied as this allowed for a 25% failure rate (not uncommon in

studies with these animals) and would still produce statistically valid data.

Split-skin grafts were covered with a synthetic dressing for periods of time up to 5 weeks. This time period was chosen as Rudolph (1979) had noted that myofibroblasts were not identified in open wounds covered with split-skin grafts in any significant numbers 35 days after grafting. If synthetic dressings were to have an effect on myofibroblast numbers then it would seem logical that this effect would take place in the first five weeks. The study was therefore designed to collect data over fifteen weeks, with the majority of measurements being made in the first 5 weeks.

The groups were identified by a letter and a number.

Group Identification.....Letter

The letter corresponded to the length of time the split skin graft was covered with the synthetic dressing, thus

Group	Time Grafts Covered with Opsite
A	1 Week
B	2 Weeks
C	3 Weeks
D	4 Weeks
E	5 Weeks

Group Identification.....Number

The number corresponded to the time (in weeks) following grafting, when the animal was sacrificed.

The following table shows that the study plan allows skin grafts on the flank and hip flexor crease to be covered

with a synthetic dressing and compared with in animal controls for specific periods of time up to fifteen weeks following operation. During this time regular detailed observations would be made of moisture vapour transmission, surface area and myofibroblast cell populations to see if these parameters are affected when the split skin graft is dressed synthetically and to see if the length of time the split skin graft is covered with the dressing influences surface area or myofibroblast measurements.

The table below illustrates how the title of the individual sub groups indicates how long the wound was covered with Opsite and when the biopsy was taken.

	Main Groups				
	A	**B**	**C**	**D**	**E**
	A1				
	A2	B2			
Sub	A3	B3	C3		
Groups	A4	B4	C4	D4	
	A5	B5	C5	D5	E5
	A10	B10	C10	D10	E10
	A15	B15	C15	D15	E15

For example:

Group A (Dressing covering the skin graft for one week)

A1 - Grafts measured and biopsied after 1 week.

A2 - Grafts measured and biopsied after 2 weeks.

A3 - Grafts measured and biopsied after 3 weeks.

A4 - Grafts measured and biopsied after 4 weeks.

A5 - Grafts measured and biopsied after 5 weeks.

A10 - Grafts measured and biopsied after 10 weeks.

A15 - Grafts measured and biopsied after 15 weeks.

Groups B,C,D, and E were similarly sub-divided. Whole sub-groups would be sacrificed when wounds were biopsied. This would eliminate the situation where biopsies and surface area measurements were regularly carried out on the same skin graft.

3.4 Skin Graft Harvesting

The presence of a panniculus carnosus in the rat allows considerable skin mobility which makes split skin graft harvesting difficult. The use of an electric or battery powered dermatome allows repeatable precise harvesting but this must be used with an anchoring device (Rudolph 1971). Techniques reported have suggested the use of a clamp but they require either an assistant or the use of genetically identical donor rats or both (Gustavson 1971, Hansen 1956, Millington 1968, Smahel 1986, Woodruff 1955).

A new method was therefore devised which:

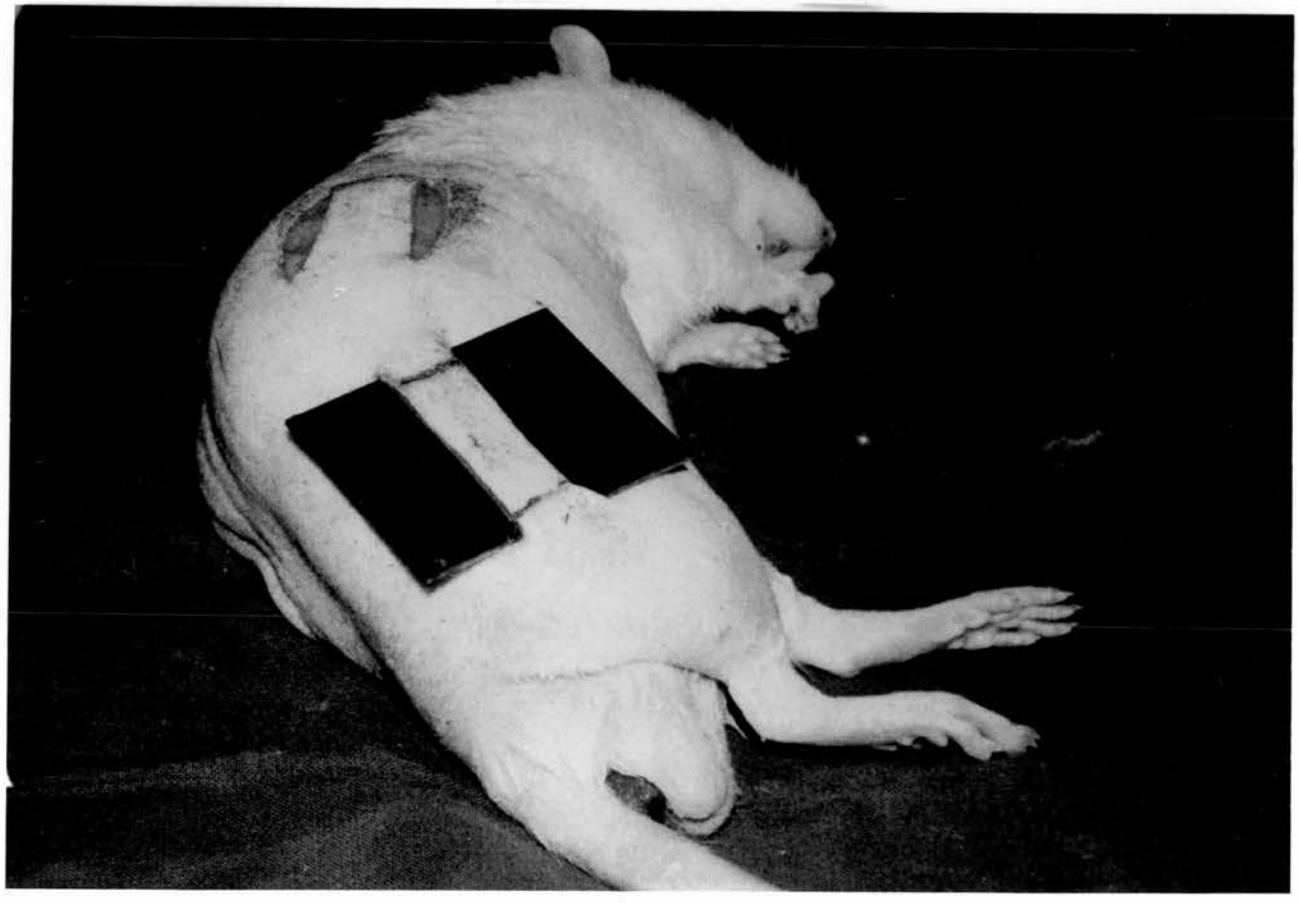
a) required no assistant
b) used the skin to be excised for the formation of the open wound as donor skin thus eliminating the need for donor animals or separate wounds on the same rat
c) required only one custom made metal sheet which took a few seconds to position to allow easier graft harvesting
d) allowed rapid graft harvesting, on average the harvesting and application of 4 split skin grafts on one rat took 20-30 minutes
e) was repeatable

Method

The rat was anaesthetized with intra-muscular Hypnorm (fentanyl/fluanisone) 0.3mg/kg. and intra-peritoneal diazepam 1.0mg/kg. The skin from behind the front legs to the tail was shaved with powered vetinary hair clippers and depillated with depilatory gel (Smith and Nephew Research Ltd.).

The areas on both flanks and hip flexor creases were marked by tracing around a 2x2 cm. square of perspex placed over these areas. Two incisions were made through the skin on opposite sides of the square, through the panniculus carnosus.

The plane under the panniculus carnosus was dissected with scissors and the flat steel plate was inserted.

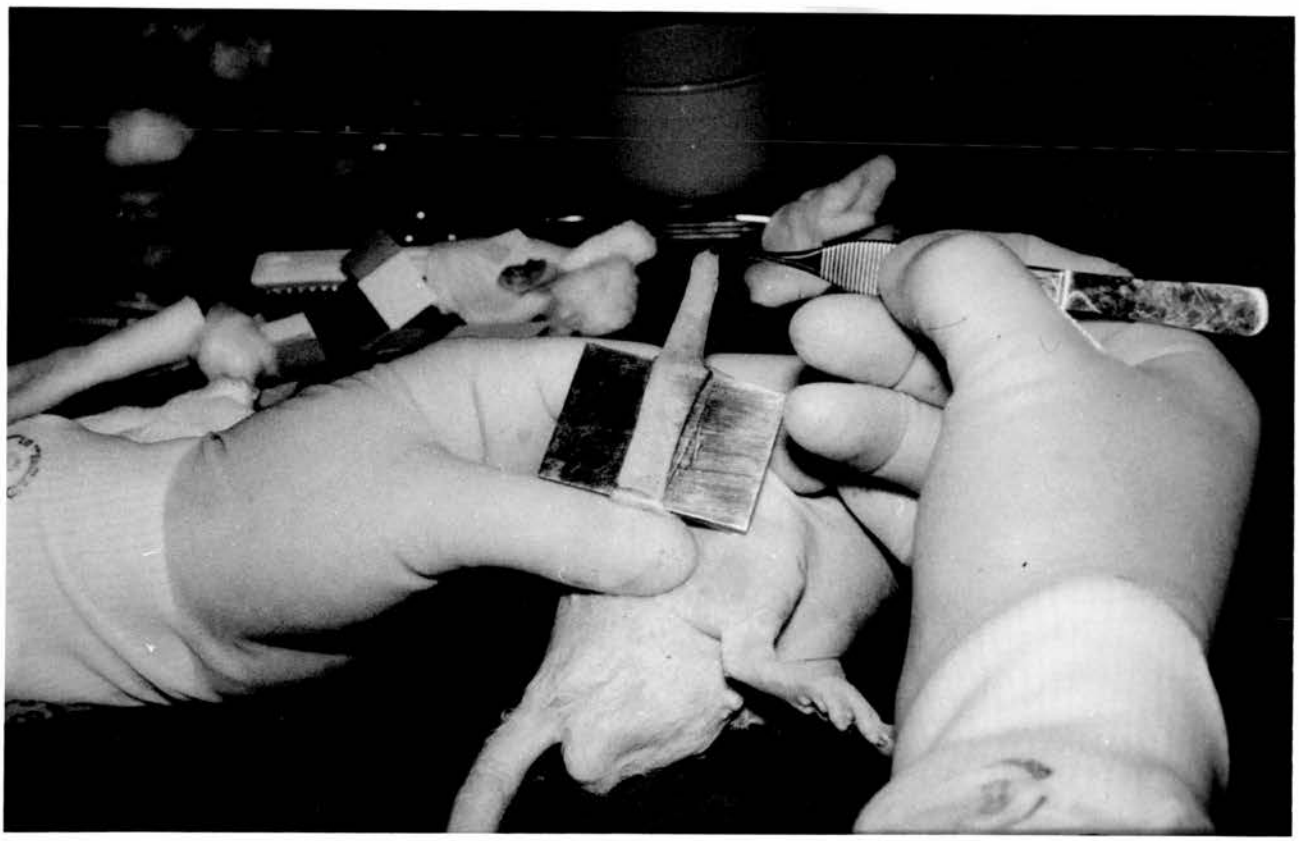


If the metal block was 1 cm. larger all round (3cm.x 3cm.) than the wound area (2cm.x 2cm.) the tension applied allowed rapid, precise graft harvesting. If necessary the incisions were minimally lengthened to allow easier plate insertion.

If the skin immediately caudal and cranial to the metal plate was grasped between the index finger and the thumb of the non-dominant hand, sufficient tension could be applied to flatten the skin against the metal plate by approximating these two digits. This tension usually resulted in the two uncut, marked edges of the square moving to the edge of the plate.

The skin was then lubricated with a little mineral oil and the graft was harvested with an electric dermatome.

Once the predetermined amount of skin had been harvested (to the previously marked fourth side of the square), the dermatome was stopped and withdrawn. This manoeuvre allowed the skin to reverse over the cutting edge of the dermatome so reducing the risk of the cutter damaging the newly harvested graft. When the skin was removed from the dermatome the attached edge was cut with scissors, freeing the graft.



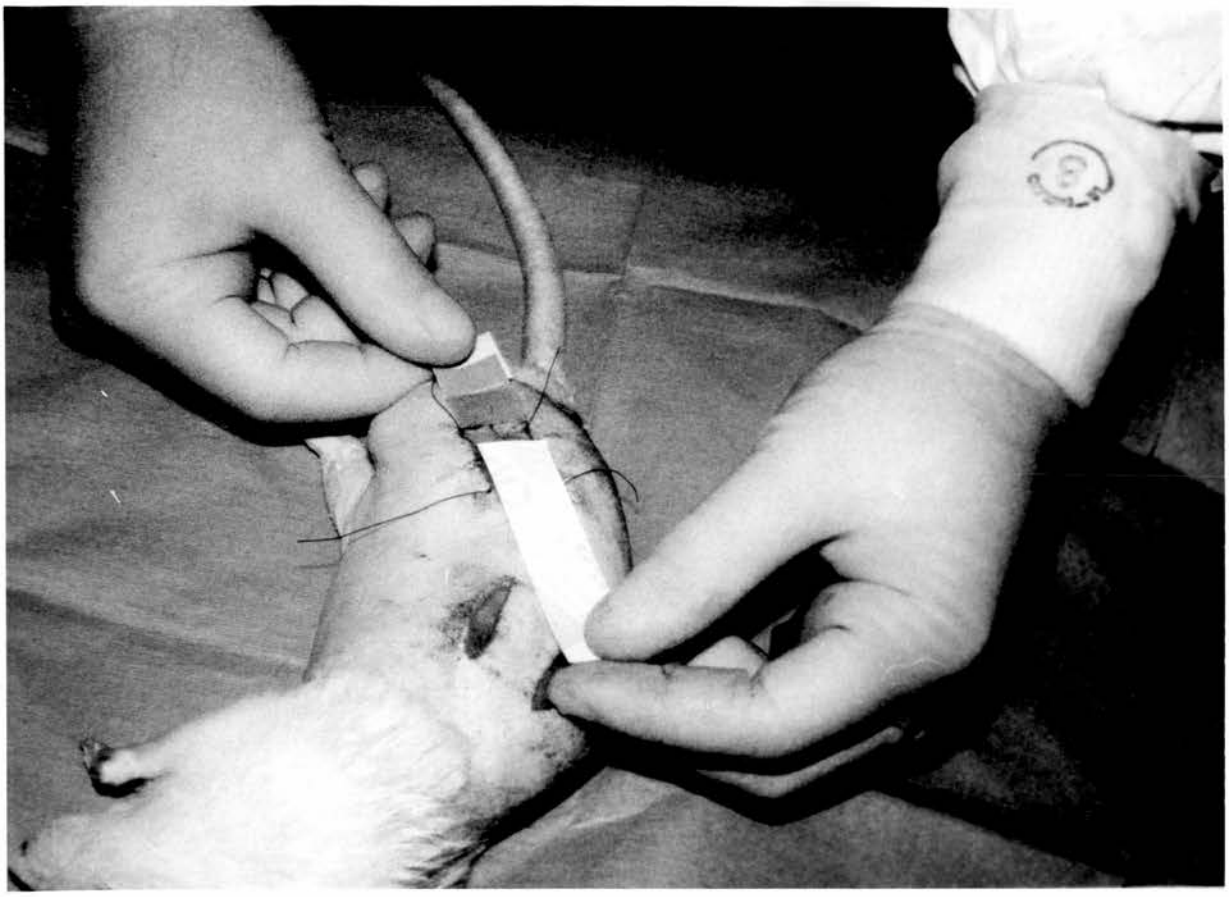
The underlying tissue was excised to the metal plate and this was removed revealing an open wound ready for skin grafting.

The skin graft thickness was measured with an electric microtome (Model Mitutoyo Digimatic Indicator) and sample biopsies were taken for histological evaluation to confirm graft thickness and the similarity of both sides (Appendix 2).

The increase in size produced by stretching the skin was lost when the skin underwent primary, physiological contraction immediately after harvesting, returning to its original size. (Grabb and Smith 1968)

The split skin grafts were sutured in situ with one 4/0 black silk suture (Ethicon U.K.) at each corner and three 6/0 nylon sutures (Ethicon U.K.) on each side of the square

The protocol was then checked and the grafts on one side of the rat were covered with the synthetic dressing (Opsite Smith and Nephew U.K.) cut exactly to size.



The relevant measurements were taken at this stage and the grafts on both sides of the animal were then dressed identically. Using the tie-over technique a small ball of cotton wool was held over the graft with the long ends of the corner silk sutures.(Brown and McDowell 1958)
The rat was then covered with a protective vest and a thoracic "waistcoat" applied to prevent the animal interfering with the dressings.
The rat was then taken to a recovery area and given an injection of 10 mls of 0.9% sterile saline solution into the dorsal aspect of the neck to compensate for any per-operative fluid loss.
When the rat had sufficiently recovered, it was taken to the main housing room and placed in an individual cage.

3.5 Moisture Vapour Transmission

Water loss or moisture vapour transmission from a wound is the factor most obviously influenced by a synthetic dressing. In open wounds water loss is in the region of 60 grammes of water per meter squared per hour (Foresman 1986). The application of a synthetic dressing markedly reduces the water loss and thus increases the humidity in the wound healing environment (Foresman 1986).

An evaluation of the water loss from a wound covered with a split skin graft dressed with a synthetic dressing compared with a similar wound without the synthetic dressing would be of interest.

The moisture vapour transmission rate (M.V.T.) from the surface of the control skin graft could also be compared with the M.V.T. rate from the surface of the skin graft covered with Opsite before and after its removal. When the dressing was removed the M.V.T. would be measured for 30 minutes post removal. This period was deemed the longest safe period to ensure animal survival. This would give an idea of the degree of humidity of the wound healing environment.

These measurements would be carried out on all sub-groups to see if the values were affected by how long the skin graft was covered with the synthetic dressing.

Procedure

The M.V.T. rate was measured with an EP1 evaporimeter (Servomed U.K.), coupled to an 8mm. diameter circular probe.

Pre-measurement Standardization

The probe was positioned over a clean dry petri dish for one minute to standardize the instrument at zero prior to beginning measurements.

When measurements were being taken the probe was held over the area in question for one minute before a stable reading was recorded.

Readings were taken when the open wounds were made, prior to the application of the skin graft, and from normal shaved skin to compare with published data (Foresman 1986).

On removal of the synthetic dressings the probe was held over the skin grafts to record the m.v.t. rates at 1, 10 and 20 minutes following dressing removal. This procedure was repeated 4 times on each animal to record the m.v.t. from each of the 4 grafts. The anaesthetic time for this procedure was therefore increased. If the anaesthetic time (to measure the m.v.t.) was greater than 30 minutes a significant number of rats required ventilatory assistance following the procedure. Thereafter no readings were taken beyond 20 minutes.

Readings

Readings were taken at weekly intervals in sub-groups A15, B15, C15, D15, and E15. This meant that the M.V.T. would be measured from skin grafts covered with a synthetic dressing for periods up to 5 weeks before its removal and thereafter up to the end of the study.

3.6 Surface Area Measurement

Accurate surface area measurements would permit wound contraction to be calculated, allowing comparison between synthetically dressed and conventionally dressed skin grafted wounds. In order to minimize error a standardized technique was used to measure the surface area.
This technique involved tracing the skin graft margins on to an acetate sheet and transferring this shape to a computer. A pilot study was carried out to gain familiarity with the equipment and thus reduce errors.

Pilot Study

Fifty 2x2 centimetre shapes were traced on to acetate sheets and photographed with a video camera. The image of the shape was transferred to a screen and this was traced with an electronic mouse. Surface areas were then calculated by the computer. The original shapes were then photographed and the surface areas calculated using the same technique. The surface areas of the tracings were compared to the surface areas of the shapes to assess the accuracy and repeatability of the technique.
The mean surface area of the acetate tracing was 372.4 mm^2 giving an accuracy of 6.9%. The mean surface area of the shape, found when it was photographed and directly traced by the computer mouse was 396.3 mm^2. This gave an accuracy of 0.9%. This figure compares well with the accuracy (0.6%) reported by Foresman (1986) who used a similar method to calculate wound surface areas.

For results in detail see Appendix 3

Main Study

Surface area was measured by placing an acetate sheet (which had been previously cleaned with 70% alcohol) over the wounds when they were first covered with the split-skin graft without the dressing. Tracings were made in indelible ink with a felt tip pen, the border between the skin graft and the surrounding, normal skin lying on the inner aspect of the line.

Measurements were made when the skin graft was applied and when the animal was sacrificed and the skin graft biopsied.

All measurements were stored on individual acetate sheets labeled with the group name and each shape was also labeled with its individual rat and wound number.

Surface Area Calculation

The acetate sheets were then photographed using a video camera. This transferred the shapes to a computer monitor. Using an electronic mouse, the inner edges of the shapes were traced and the surface areas calculated by the computer. The computerized results were expressed in square millimeters to two decimal places.

When the results of the individual animals in the sub-groups were added together for statistical analysis the figures were rounded to the nearest whole number. There were approximately a total of 1,600 surface area measurements taken during this study.

3.7 Myofibroblast Cell Estimation

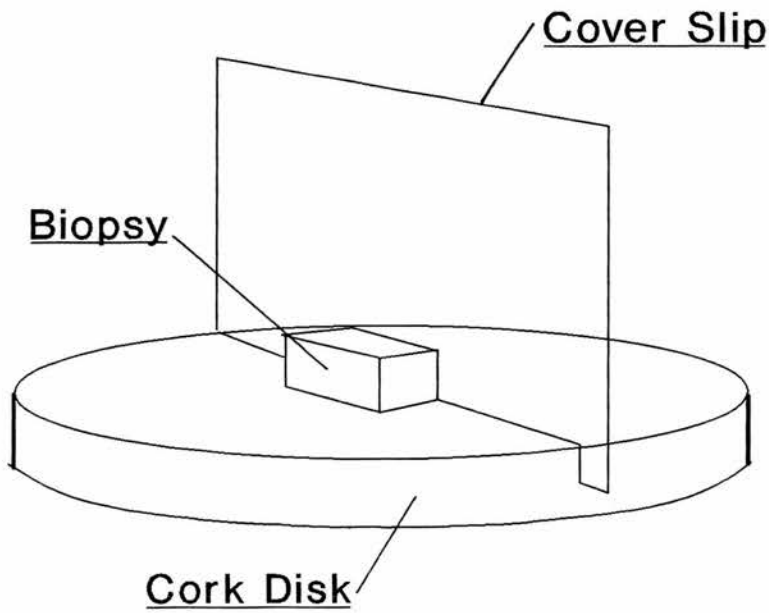
This part of the study was carried out in the Department of Experimental Dermatology, The London Hospital. Myofibroblasts are identified morphologically or immunocytochemically. Morphological analysis requires experience with electron microscopy both in specimen preparation and in the identification of myofibroblasts in the final image. This experience is not required when immunocytochemistry is used. The technique used in this experiment utilised the binding of anti-smooth muscle actin antibody to the intracytoplasmic actin filaments of the myofibrolast. This facilitated the accurate identification and quantification of myofibroblasts using light microscopy. Biopsy specimens were also larger so increasing the likelihood of the cell density in the specimen representing the population under the skin graft. This technique has proved successful when used to quantify myofibroblasts in open wounds in other studies (McGrath 1982).

Wound Biopsies

The rats were sacrificed and the skin grafts were biopsied at times determined by the study protocol. Particular care was taken to include the filamentous epimysium over the muscle under the skin graft as according to Rudolph (1979) it is in this area (immediately under the skin graft) that myofibroblasts are to be found.

Biopsies of approximately 5x5mm. were taken from the middle of the graft. This area was chosen as this part of

the graft was closest to the area of the skin graft that had been assessed for thickness. The biopsies were oriented on a cork disc the underside of which had been previously marked with the wound and rat number. Orientation was carried out with the aid of a glass cover slip so that a transverse section of the graft and the underlying structures were uppermost, lying parallel to the disc as can be seen in the drawing below.



This would aid the sectioning procedure considerably. A small blob of fixative gel was then applied, covering all the tissue. The whole disc and attached tissue was then cooled by immersing it in liquid nitrogen. The disc was removed and the cover slip warmed. This allowed the cover slip to be removed from the disc leaving the attached, orientated specimen behind. The exposed portion of the specimen was then covered with fixative and the disc was returned to the liquid nitrogen.

All the biopsies from a sub-group were then collected and placed in a labeled plastic bag. These bags were then stored in the in-house -70°C refrigerator. They were then transferred, in liquid nitrogen to the Dept. Experimental Dermatology The London Hospital where they were placed in plastic boxes labeled with the main group letter. All boxes were then stored in a -70°C refrigerator until sectioning was undertaken.

Sectioning

This process involved controlled thawing of the biopsies to -20°C and cutting eight sections per biopsy. This number of sections were needed to allow both positive and negative controls to be used at various stages of the staining procedure. The labeled biopsies were sectioned and at least 8 sections per biopsy were taken and placed on a single glass slide. The glass slide was then labeled and returned to the refrigerator until staining was carried out.

Immunoperoxidase Staining

Sections were recovered from the -70°C freezer and air dried in a flow Calasef hood for 15 minutes.

The alpha-anti smooth muscle antibody was added to cover the whole section. This preparation was then incubated for one hour at room temperature in a humidification chamber. The sections were then washed in physiologically buffered saline three times (5 minutes each wash). The sections were then incubated with Rabbit anti-mouse peroxidase conjugated serum for a further hour. The washing procedure was then repeated, with the last wash containing D.A.B.. The D.A.B. (3,3'-Di-aminobenzidine with 0.1% hydrogen peroxide) substrate was made up during the washing periods and was thus fresh when applied to the sections. When the tissue began to turn brown the sections were washed and a counter stain of haematoxolin and eosin was applied to assess tissue architecture. The section was then mounted with a cover slip and the myofibroblast cell clumps counted. Results are expressed as numbers of cell clumps per unit surface area (of the skin graft).

Data from similar wounds treated in a similar way in each group was accumulated. Mean values and 95% confidence limits for the mean were then calculated. Paired Student's "t" tests were then carried out comparing the results in the control groups with those in the synthetically dressed groups.

CHAPTER 4

Results

4.1 Failures

4.2 Rat Weights

4.3 Moisture Vapour Transmission

4.4 Surface Area

4.5 Myofibroblasts

4.1 Failures

Definition

A failure was defined if the animal unexpectedly died at any part of the study, or when the skin graft had been damaged in any way other than in a superficial manner. This was defined as there being enough trauma to the graft to cause bleeding or serous ooze from the area of damage. Perhaps surprisingly, in view of this animal's reputation for disruption, there were fewer than anticipated failures.

Failure rates are given under the following headlines;
Animal death both per and post operatively,
Animal interference and skin graft failures, and
Technical failures in the three experiments

ANIMAL DEATH

Per-Operative Deaths

Only three rats died per-operatively (1.5%). This extremely low figure was achieved because of several factors.

1) The considerable experience of the Animal house staff in handling and anaesthetising rats

2) The devising of a new technique which allowed the rapid harvesting and application of 4 skin grafts on one animal

3) Two pilot studies were carried out before the main study to allow the operator to become experienced in the necessary techniques. The first study involved dead animals and the technique of harvesting skin grafts was practised. Once proficiency had been gained the technique was used on anaesthetised rats to work out exact drug dosage, to increase the speed of the operation and to es-

tablish the best dressing techniques.

The three animals were replaced on the days of the wounding thus restoring the group numbers.

Post operative Deaths

Only one rat died (No.1283) in the post-operative period. This animal lost weight in the immediate post-operative period which was a normal occurance in all rats but failed to gain weight over the following three weeks. It died four weeks after the operation having been excluded from the experiment (and thus having all dressings removed) two weeks beforehand. Its position in the study was not replaced reducing the numbers in the sub-group (D15) to 7, which still allowed statistical comparisons to be made.

Animal Interference and Skin Graft Failures

The ammount of grafts subsequently excluded from the study ammounted to 57 grafts.

The reasons for exclusion were:

Operative Complications

One skin graft was applied to the wound incorrectly. The outer aspect of the graft was applied to the wound instead of the inner aspect, this resulted in total failure of the graft. It was therefore excluded from the study.

Post-operative Complications

Out of 800 skin grafts applied there were only 3 instances when a haematoma (1 graft) or a seroma (2 grafts) caused graft failure. This extremely low figure is even more surprising when one considers that no formal method of haemostasis was used when the grafts were harvested and applied to the open wound.

Rat Interference

The reason for the exclusion of the remainder of the grafts (53 in total) was animal interference. The rat interfered with the graft in usually one of two ways.

Gnawing

This only happened to the grafts over the hip flexor crease as the anatomy of the rat dictated that it could not reach its flank with its teeth. A protective vest was placed around the cervical region to prevent the rat damaging the dressings over the hip flexor area. Despite this three rats were able to free themselves from the collar and damage the (6) grafts on their hip flexor creases.

Scratching

Following the removal of the tie over dressings the rat was dressed with a toweling "waistcoat" to prevent it scratching the skin graft on the flank. This dressing was well tolerated by the animals but the tension required to keep the vest on the animal was critical. If it was too loose the animal was able to work its way out and subsequently damaged the grafts, if it was too tight the animal became distressed, which was unacceptable. Therefore the dressings were applied to fit in a loose manner. This was the main reason for the majority of exclusions (47 grafts) from the experiment. This was felt to be an acceptable failure rate to guarantee animal comfort.

SUMMARY

Rat Deaths

Per-operatively.....3 (all replaced)

Post-operatively.....1 (not replaced)

Grafts Excluded from the Study

Graft Failure.....1

Haematoma.....1

Seroma.....2

Animal Interference.....53

Technical Failures in each Experiment

Moisture Vapour Transmission

There were 160 skin grafts studied (40 rats with 4 skin grafts per animal). There were four grafts not measured, all on one rat which died (Gp.D15, Rat No.1283). The failure rate on this part of the experiments therefore was 2.5%. No rats in these groups damaged any part of their grafts.

Surface Areas

There was no additional failures associated with the technical transfer of the wound surface areas from the rats to the computer.

Myofibroblasts

There were no failures in this part of the study as the techniques of sectioning, mounting and staining were practiced beforehand. If any errors were made in the main study there was enough spare biopsy material to refer to for further processing. As a result of this, all specimens

were processed giving results which are tabulated below.

OVERALL FAILURE RATE

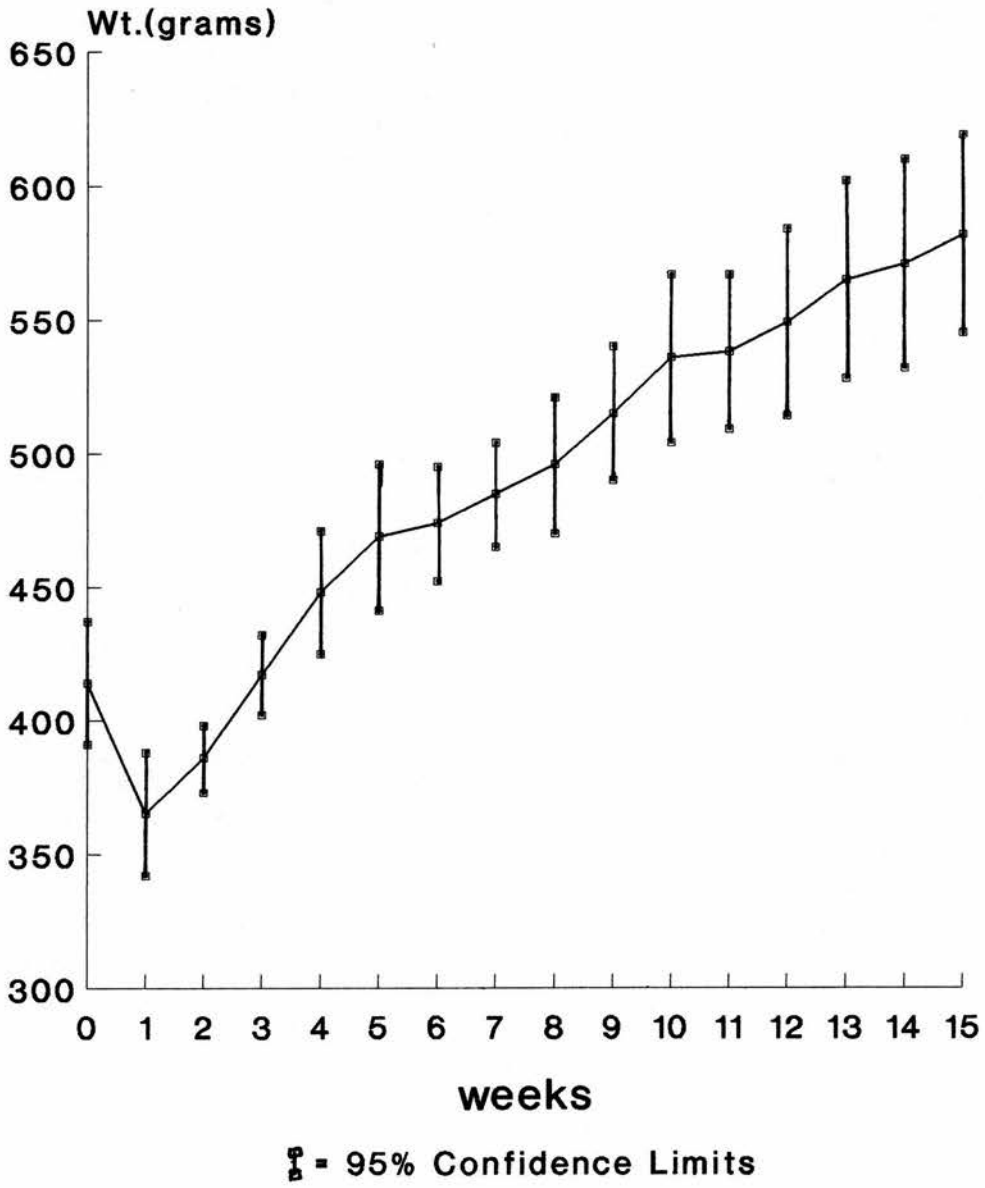
A total of 61 grafts were excluded from the study [1 rat death (4 grafts) and 57 grafts] of 800 grafts.
This gives an overall failure rate of 7.625%

4.2 Rat Weights

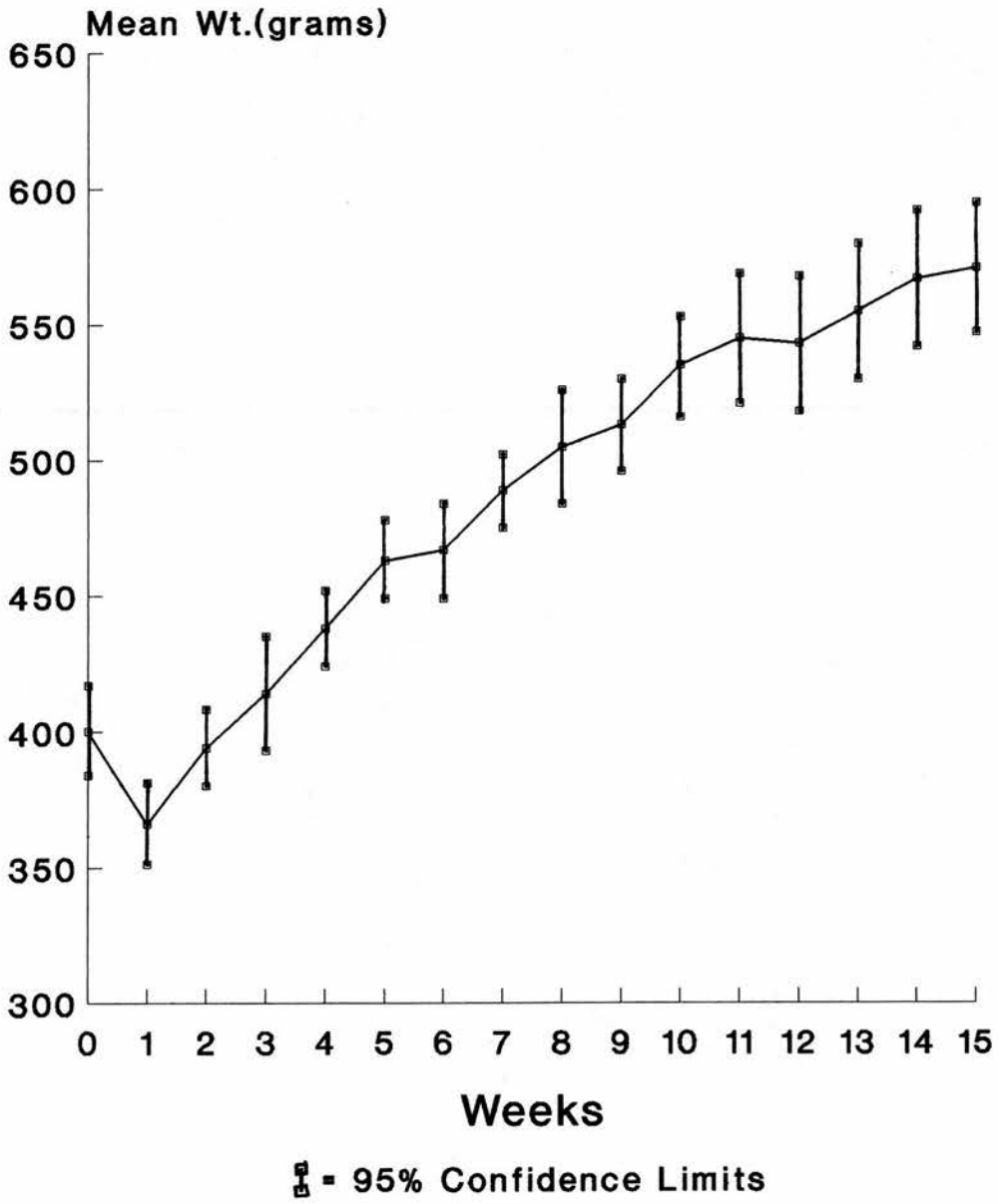
The surface area of the rat is related to it's weight (Surface Area [mm2] = 10 x weight[grams]$^{2/3}$) therefore all rats were weighed prior to entry into, and at weekly intervals throughout the study. This parameter was taken as a measure of the general well being of the animal and as a baseline measurement to indicate the similarity of each group.
The rats were weighed using a mitsuyisu electronic weighing machine which was standardised on a weekly basis. Weights were expressed in grams and the values of each group were tabulated. These tables were then statistically evaluated and the mean weight and 95% Confidence Limits for the mean were calculated for each group at weekly intervals of the study. These tables can be found in Appendix 3.
Data from the same post-operative dates was collected from each table to give values for the rat population in the main groups (A,B,C,D,and E.).

Mean group weight with 95% confidence limits (error bars) (Armitage and Berry 1987) is displayed in the following graphs.

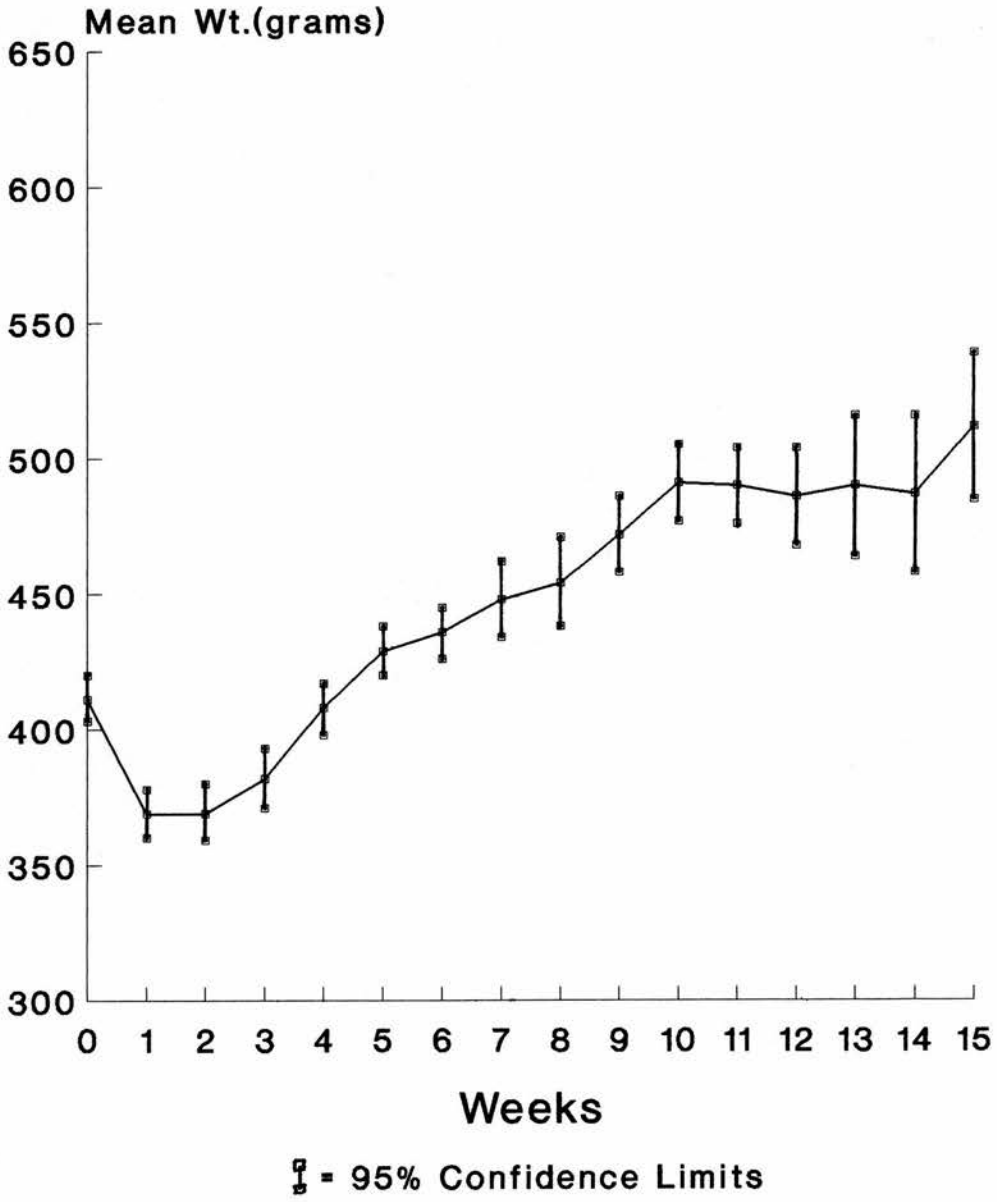
Group A
Mean Weight



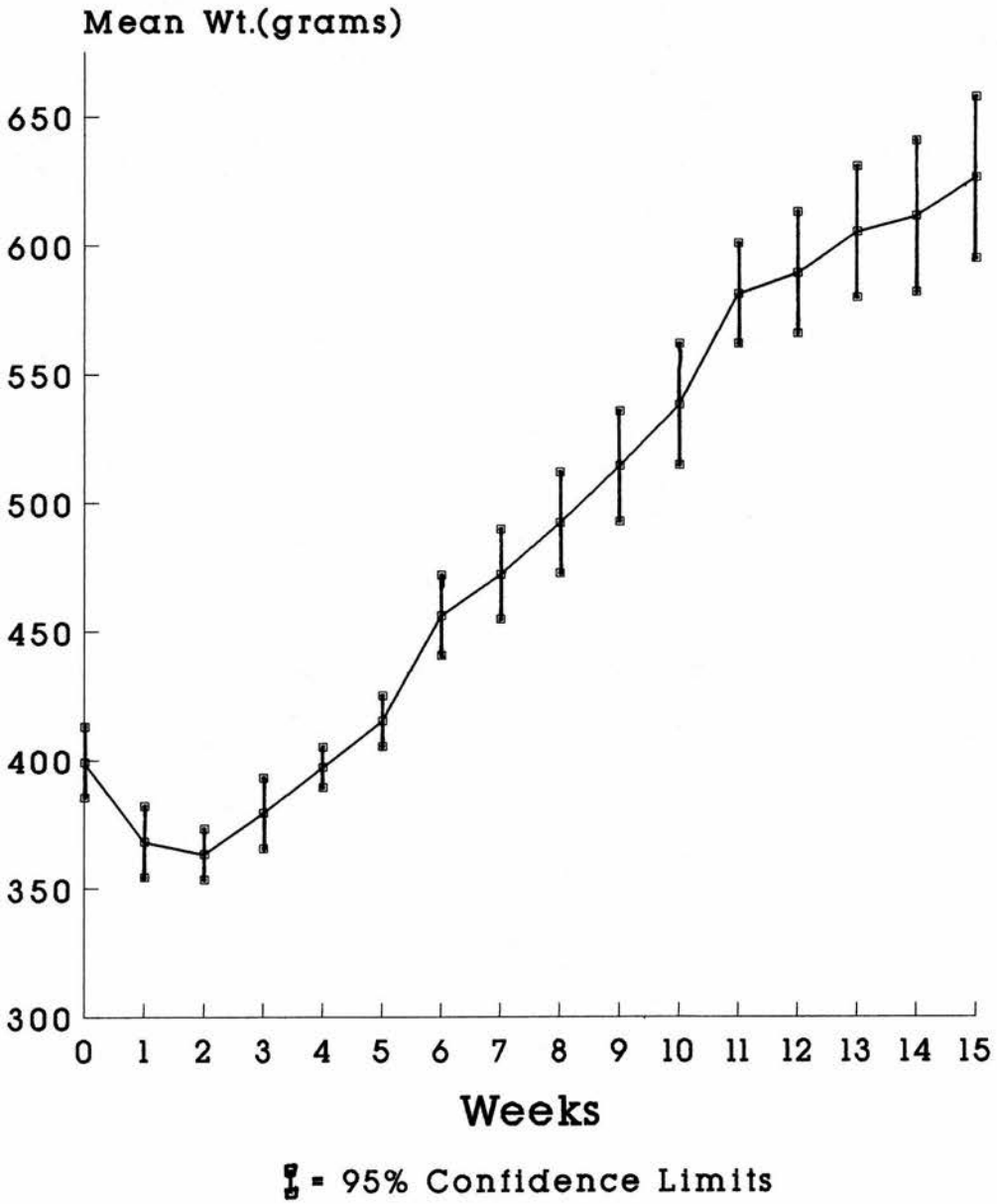
Group B
Mean Weights



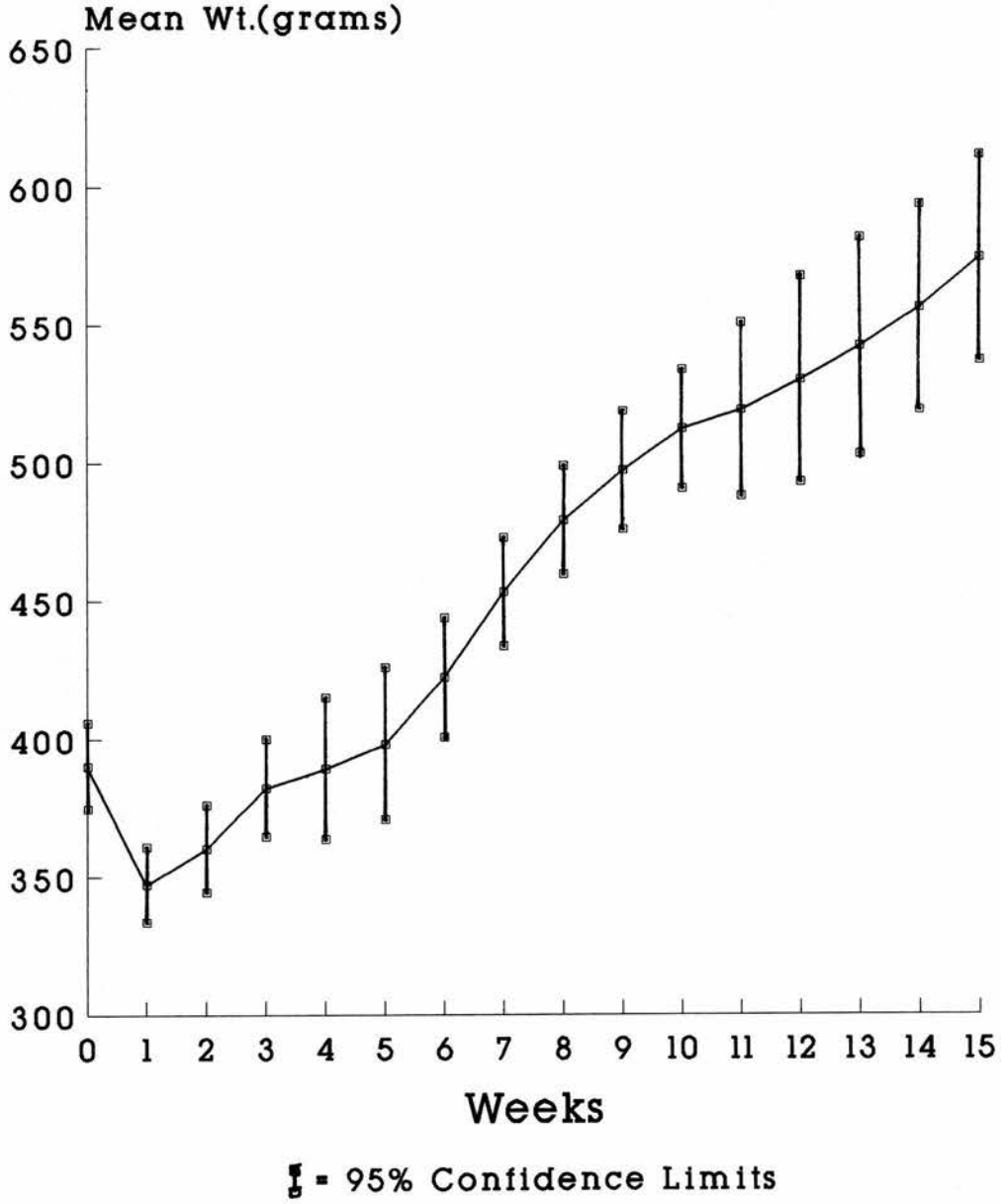
Group C
Mean Weights



Group D
Mean Weights



Group E
Mean Weights



Mean weight on entry to the study was 404g (391-418g 95% Confidence Limit).

In the immediate post operative period all rats lost weight. This phenomenon occurs after any animal experiment of this magnitude (Personal Communication, S+N Research Animal House Manager), but within 1-2 weeks the animal was gaining weight. It usually took another three weeks however for the animal to reach the pre-operative weight. It is perhaps not surprising that all animals lost weight. Before the operation the rats were housed together and had no restraining dressings. After the skin grafting procedure all rats were placed in a restraining vest with an underlying tubigrip support and then housed in individual cages. One week later (under gaseous anaesthesia) all dressings were removed, the skin grafts were checked, and lighter dressings applied allowing more mobility. The restrictive dressings and the individual housing of this social animal may have accounted for the post operative weight loss.

Thereafter, all animals increased in weight to the end of the study. Mean animal weight at 15 weeks was 573g (522 - 623g 95% Confidence Limits)

Summary

If weight is an indication of how stressed an animal is then this baseline data suggests that all groups reacted in a similar way to the trauma of the procedures. As surface area is influenced by animal weight these figures will be taken into account when the split skin graft surface area results are presented.

4.3 Moisture Vapour Transmission

Pilot Study - Baseline Measurements

To enable the results to be compared with published data, readings were taken from shaved normal skin and open wounds prior to covering them with the split skin grafts. The mean value was found to be 7.59 grammes per meter squared per hour for the shaved skin and 70.93 grammes per meter squared per hour for open wounds on the flank and a mean value of 6.87 G.M.H. for the shaved skin and 72.89 G.M.H for the open wounds on the hip flexor area. (Refer to Appendix 3 for details of this pilot study)
These values are similar to published data detailing evaporative water loss from normal skin (Lamke 1977, Jonkman 1989) and open wounds (Foresman 1986).

Moisture Vapour Transmission - Flank Area

Group A

The control grafts in this group lost water initially at a rate of 30g\m^2\hr. Over the next 4 weeks the m.v.t. fell to a value of 15g\m^2\hr. It then slowly fell to a level of 12g\m^2\hr at 15 weeks. Synthetically dressed skin grafts lost water at a rate of 15g\m^2\hr in the first week.
When the dressing was removed there was a massive surge in the moisture vapour transmission rate. This surge peaked at about 1 minute after dressing removal at 105g\m^2\hr. and thereafter fell over the next 30 minutes to a mean level of 25g\m^2\hr. At this time it was deemed unsafe to further anaesthetise the rats so they were woken up. The following day the same rats were anaesthetised and further

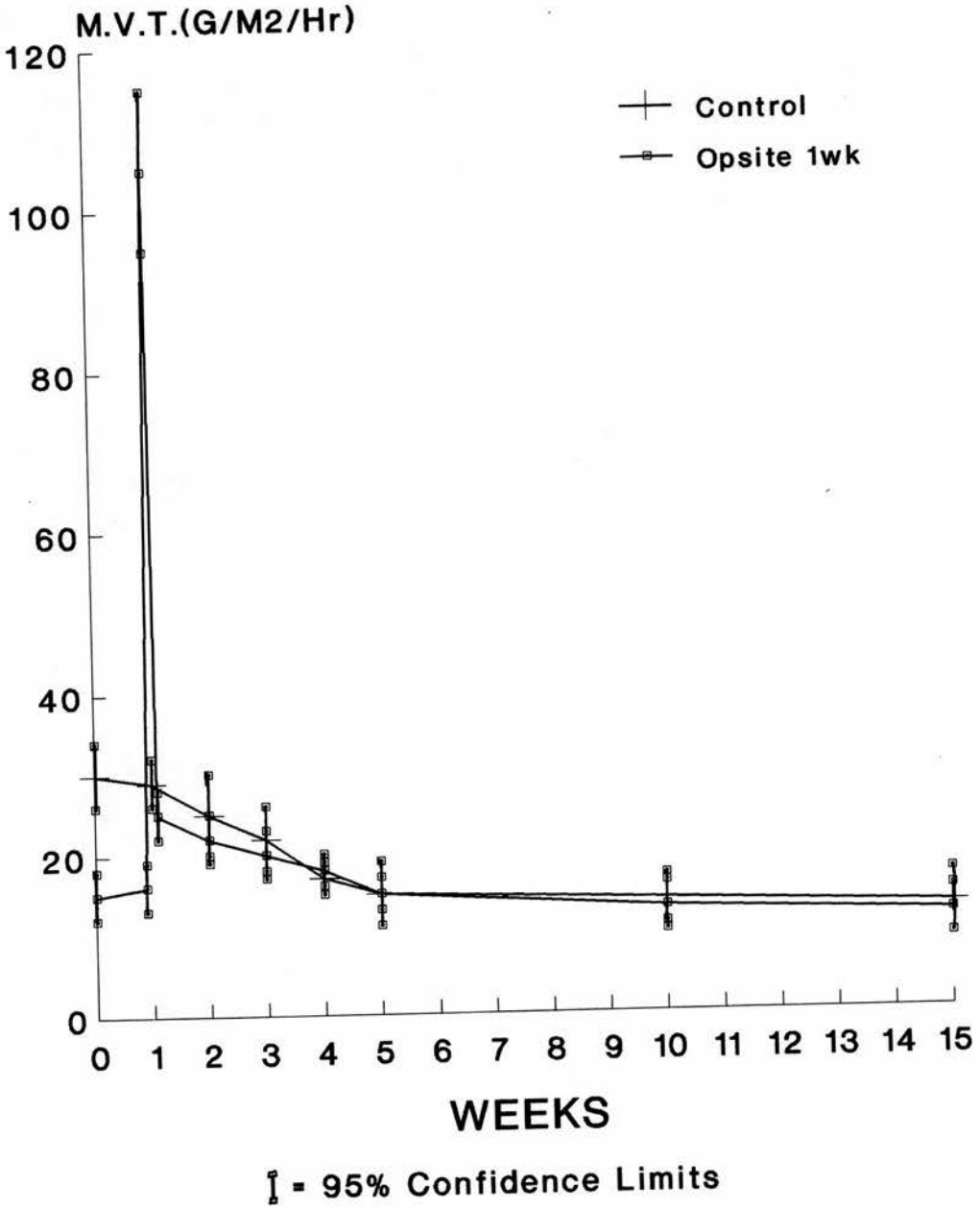
readings were made. These readings were not statistically different from the control grafts. Thereafter, skin graft m.v.t. did not differ significantly in either group.

Groups B,C,D and E.

The skin grafts in these groups reacted in a similar way to the grafts in Group A. The initial values in all control skin grafts was approximately 30 g\m^2\hr with the opsite covered grafts having a m.v.t. of approximately 15 g\m^2\hr. There was a similar m.v.t. surge when the dressing was removed. The peak of this surge was similar in all groups at between 100-110 g\m^2\hr. Within 30 minutes of dressing removal the m.v.t. in all groups was approaching that of the control group. Thereafter there was little difference in the m.v.t. of both control and opsite covered grafts to the end of the study.

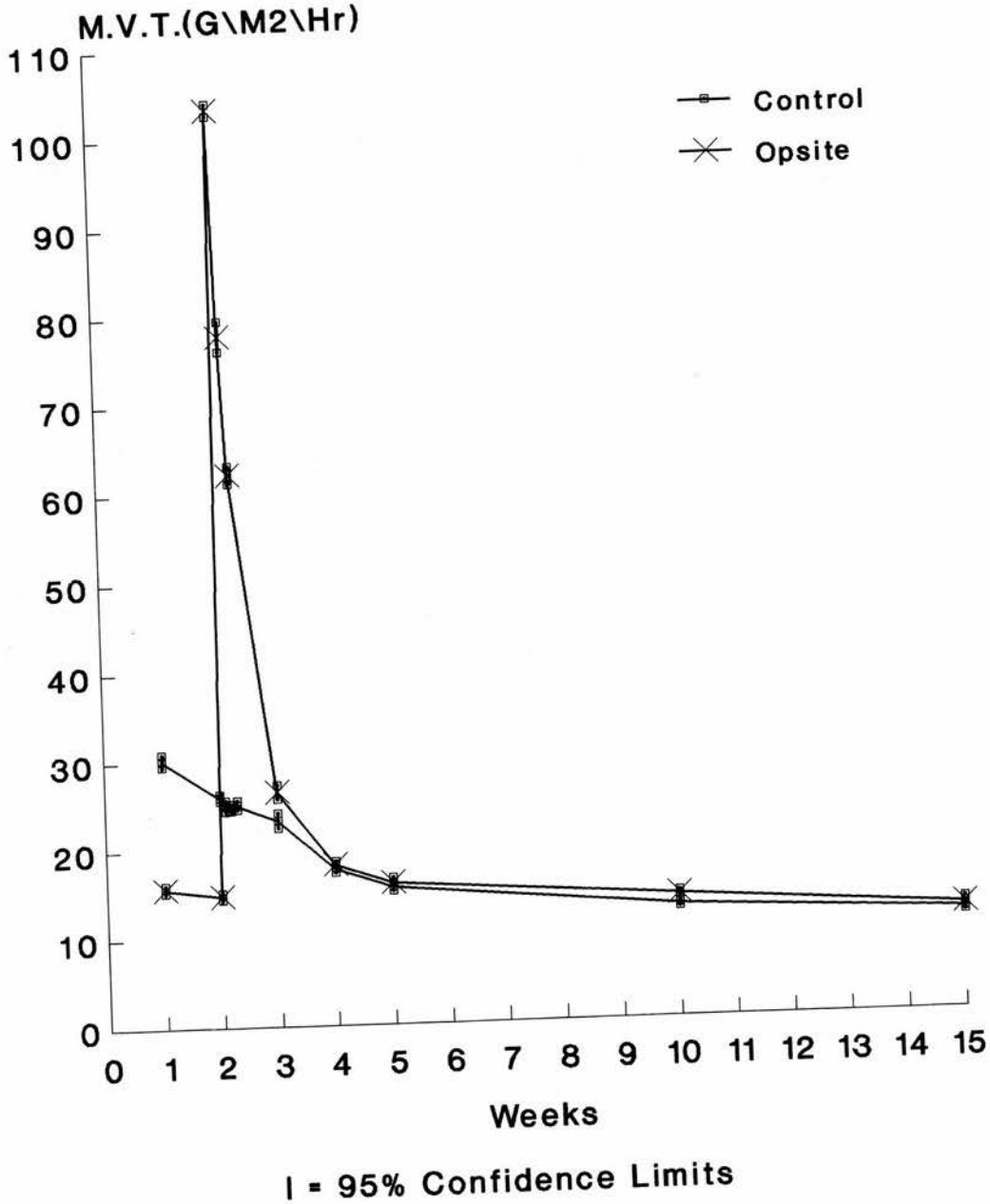
The following graphs depict mean group moisture vapour transmission with 95% confidence limit error bars.

Group A15



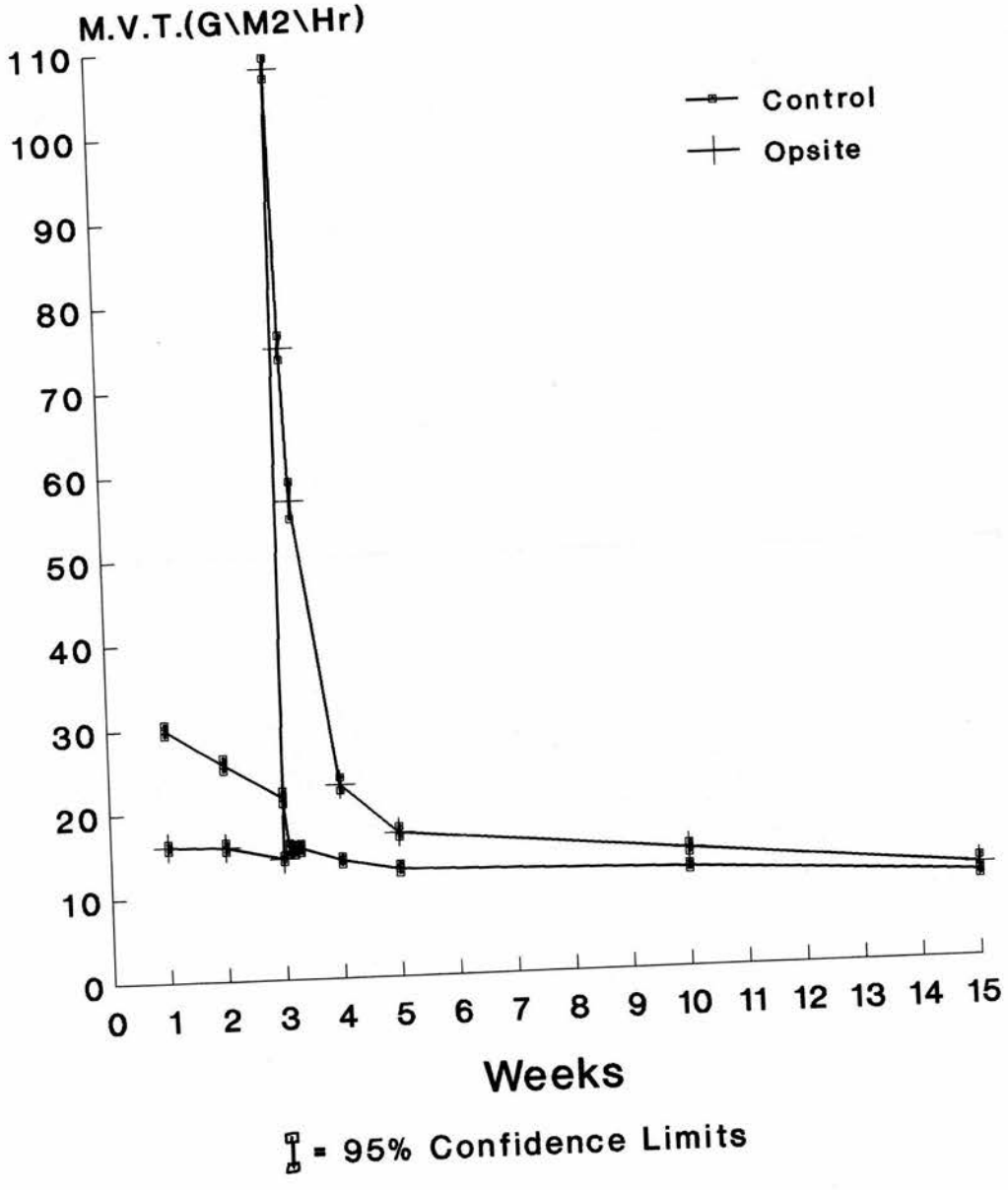
I = 95% Confidence Limits

Group B15

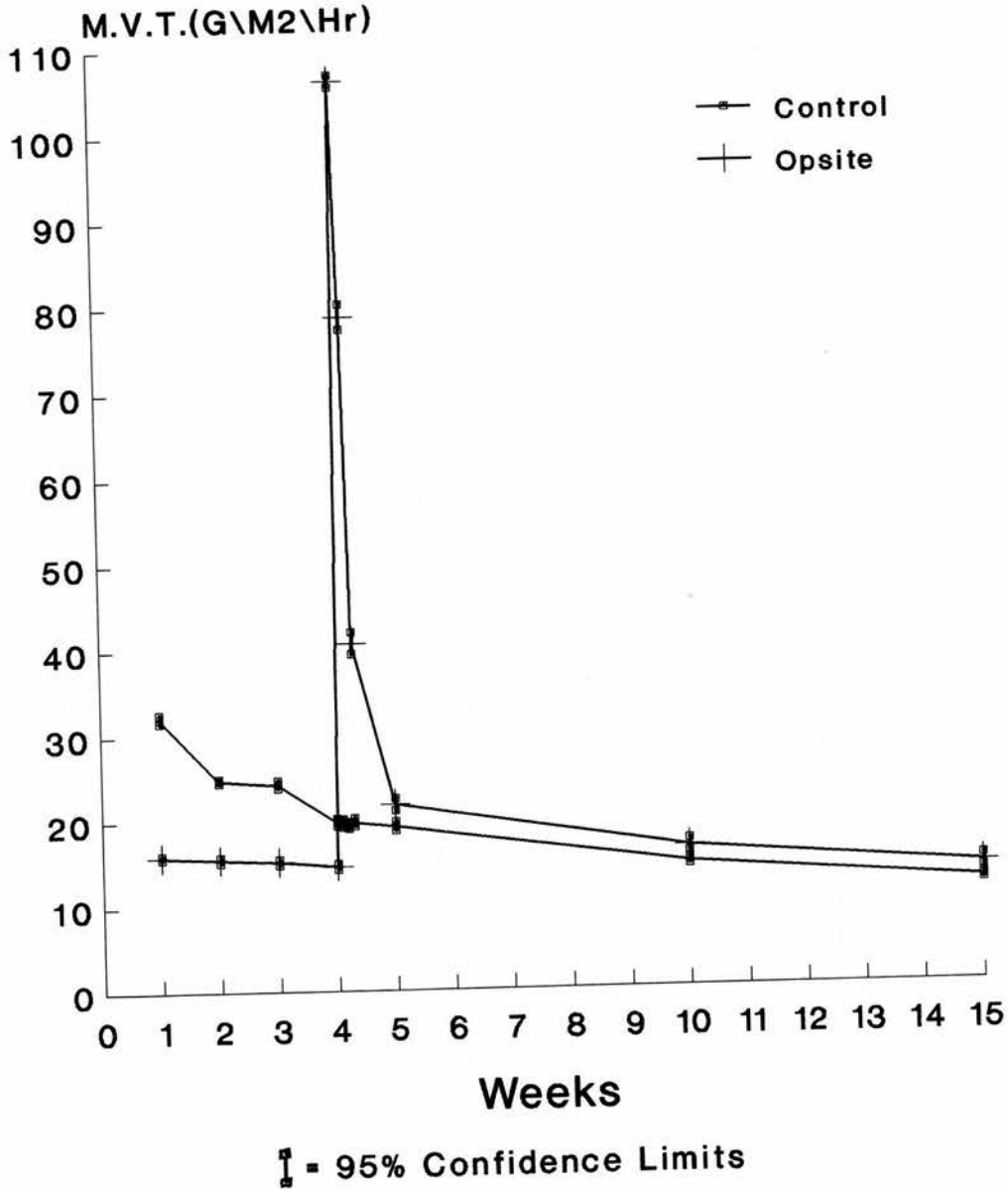


I = 95% Confidence Limits

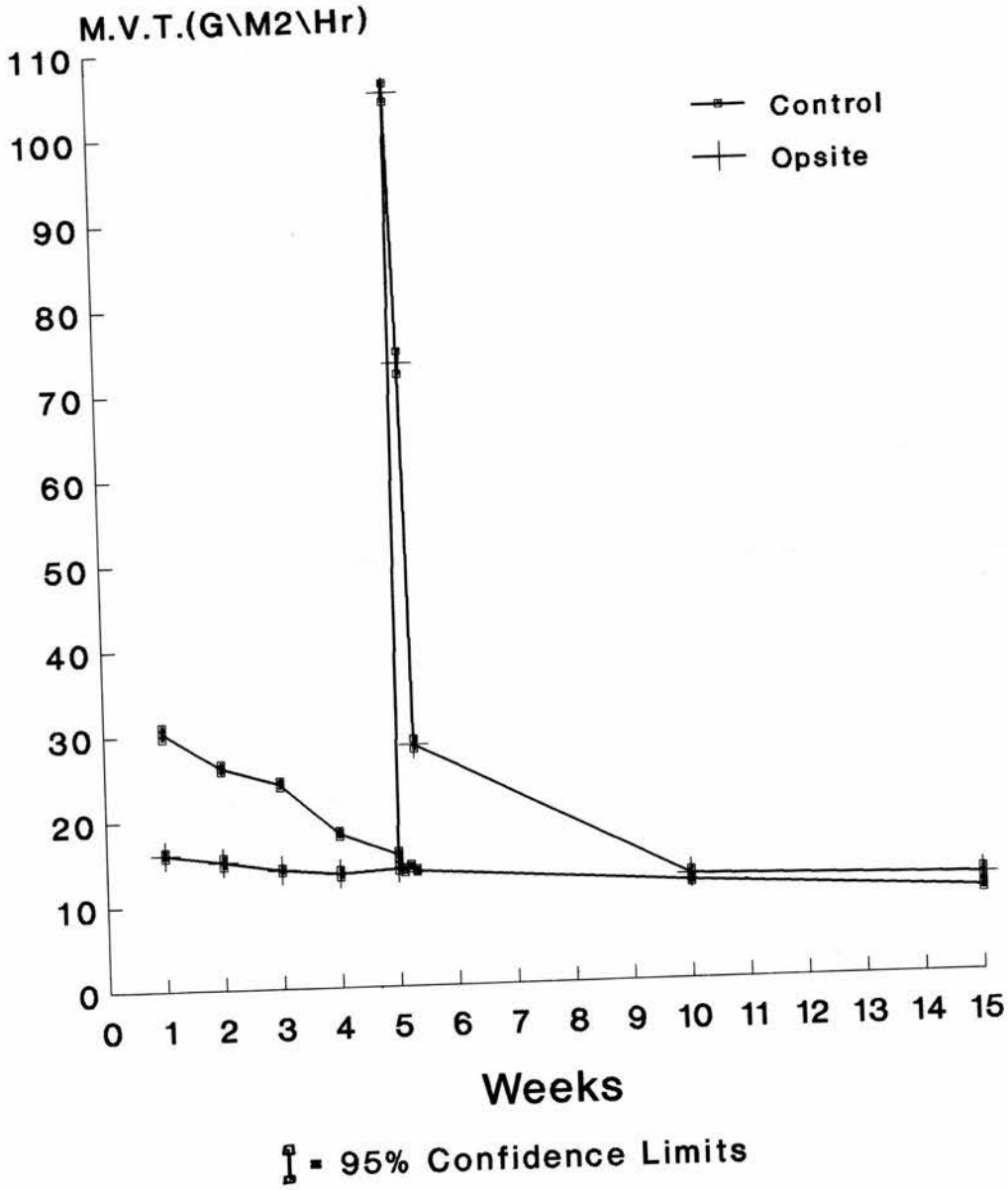
Group C15
Flank



Group D15
Flank

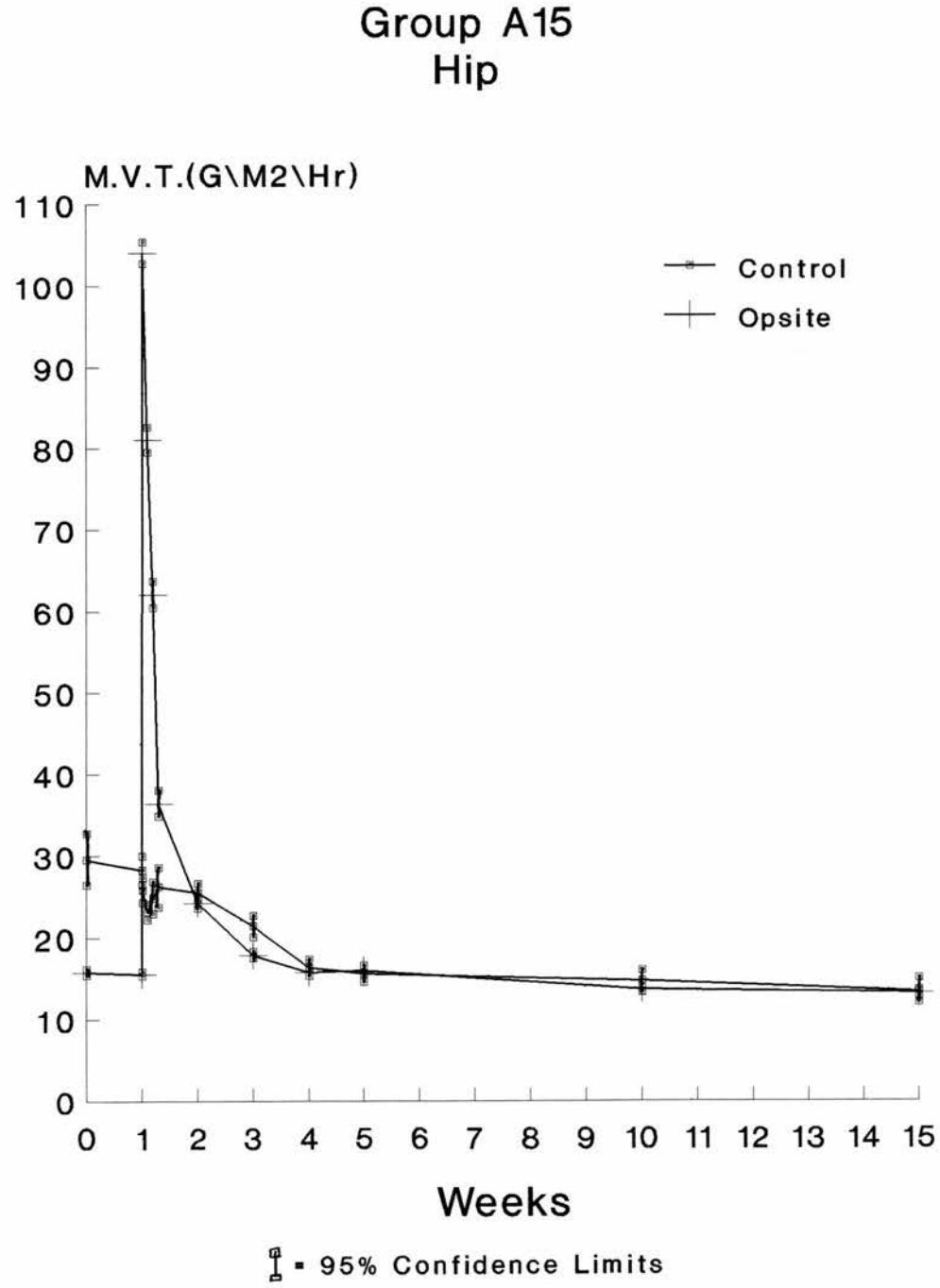


Group E15
Flank

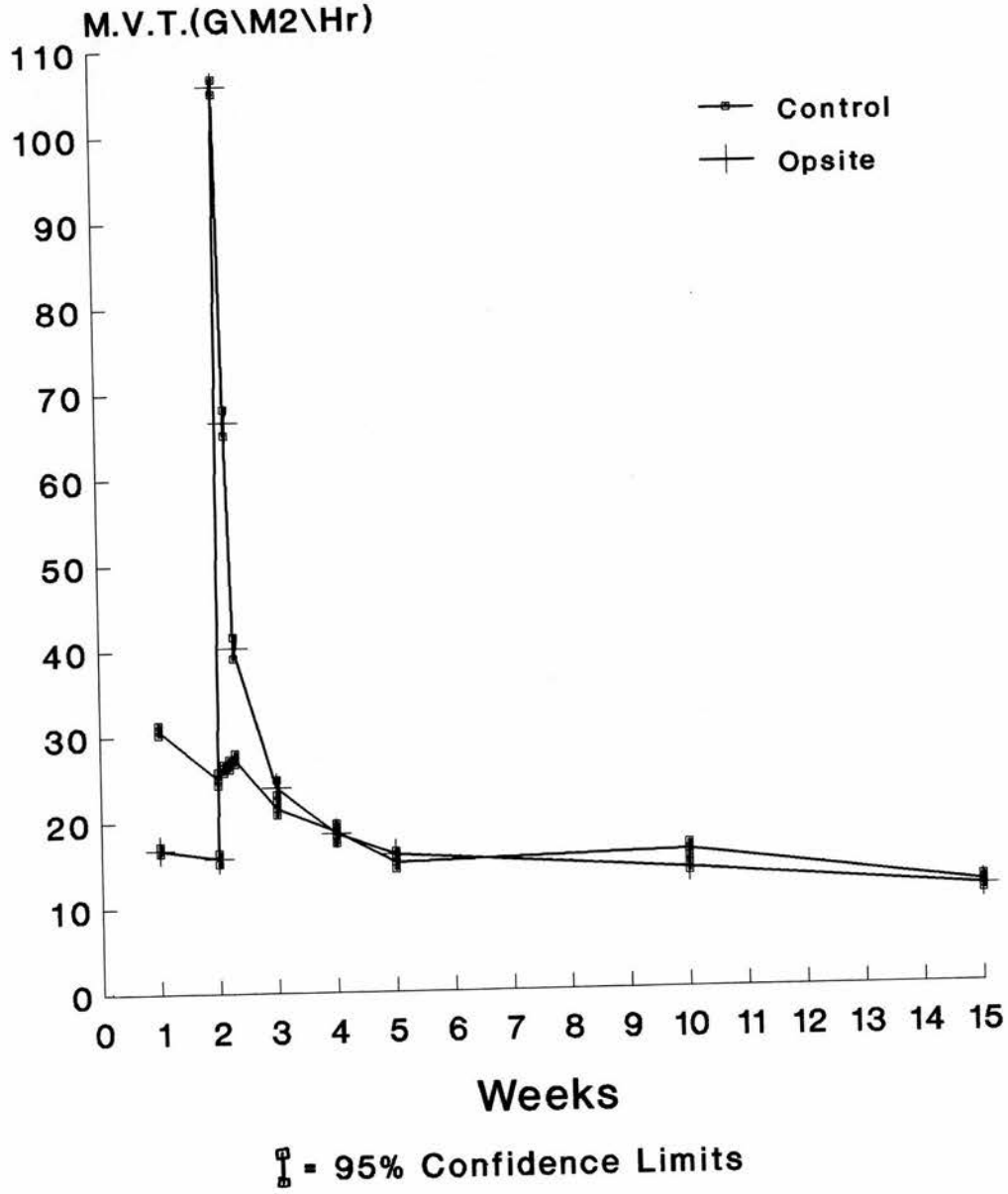


Moisture Vapour Transmission - Hip Area

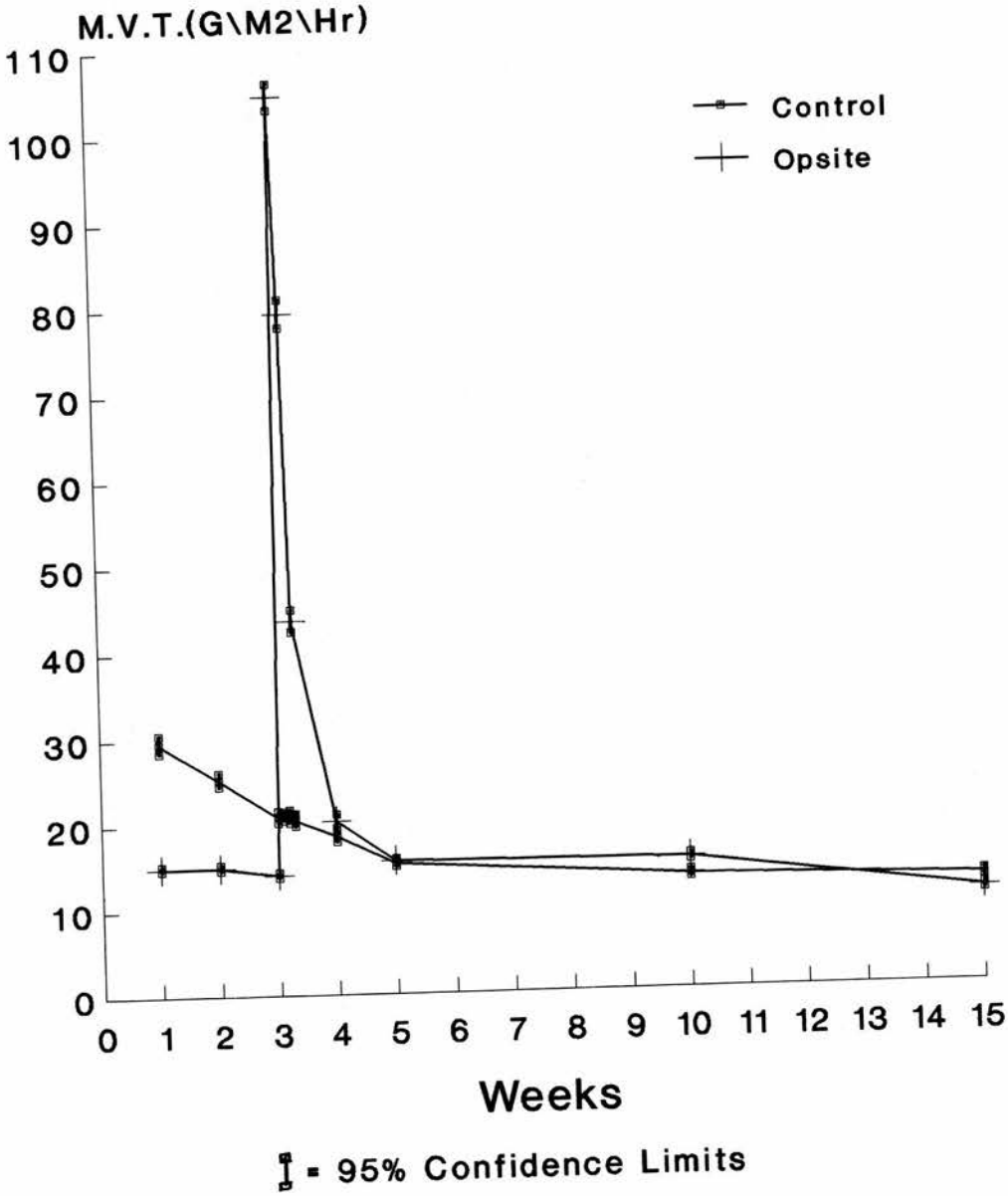
The following pages graphically illustrate the results of the moisture vapour transmission rates from the split skin grafts on the hip flexor region. The hip measurements were made at the same time as the flank observations during the same anaesthetic.



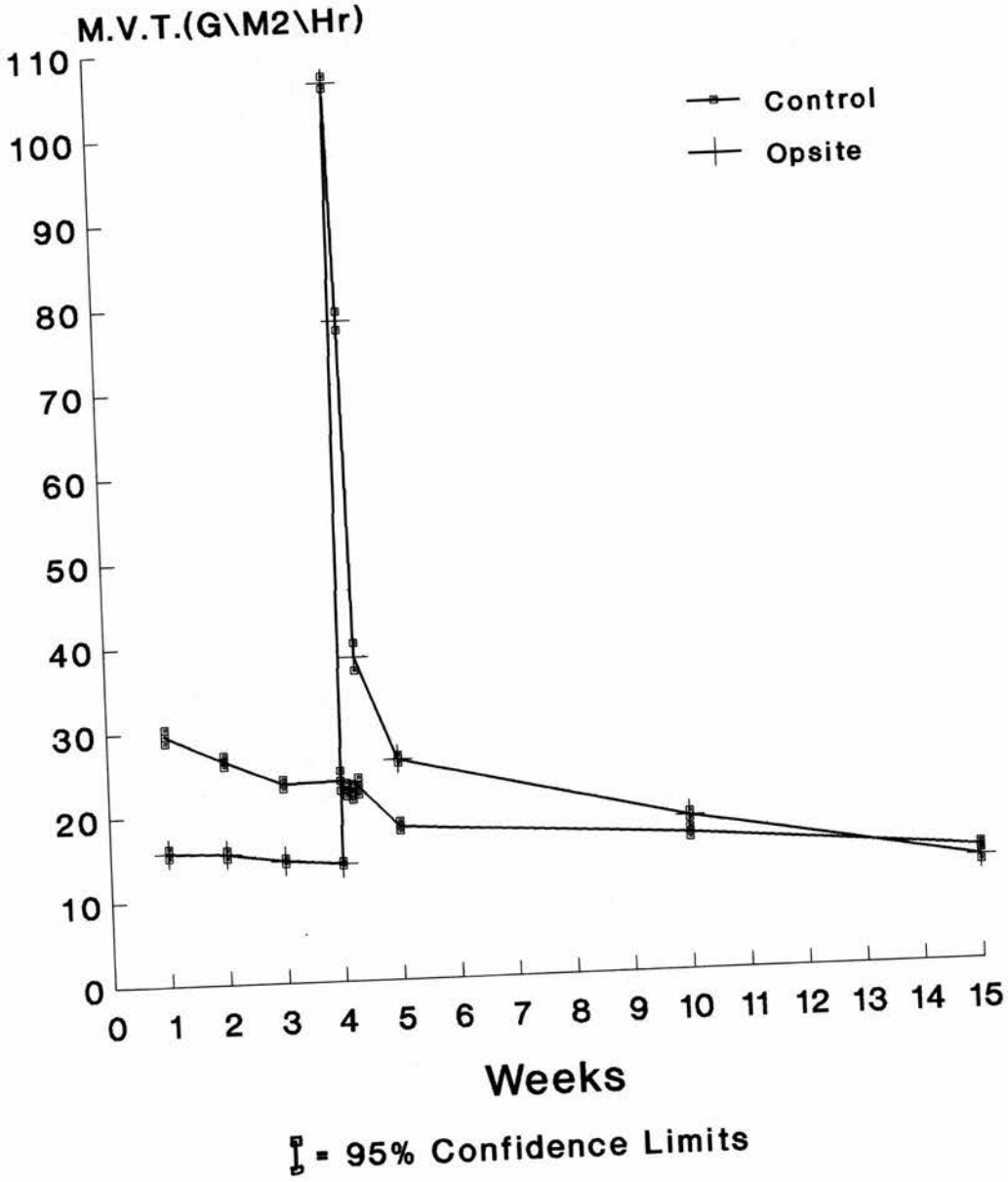
Group B15
Hip



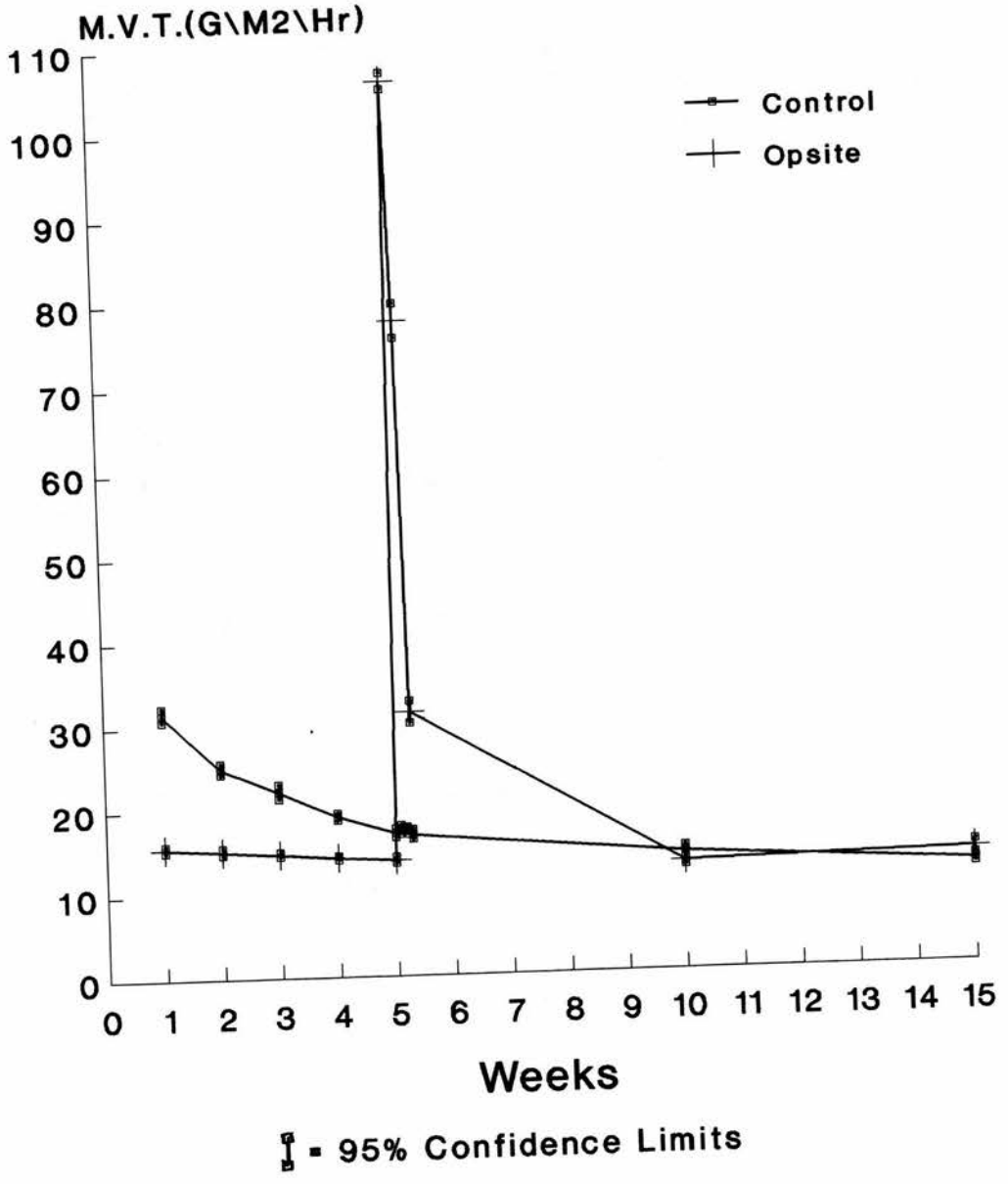
Group C15
Hip



Group D15
Hip



Group E15
Hip



Summary

Open Wounds

Mean water loss from the flank and hip open wounds in this study is 72 +/- (95% Confidence Limits) 3 $g/m^2/hr$. This is similar to the values recorded by Foresman (58 +/- (Std.Dev.) 6 $g/m^2/hr$).

The narrow 95% confidence limit bars are probably the result of the environmental controls which stabalised the light, temperature and humidity in all the rooms used during the experiments (See Appendix 1).

Split Skin Grafted Wounds

Covering the wound with a split skin graft reduces the Moisture Vapour Transmission (m.v.t.) to 30 $g/m^2/hr$. Over the next few weeks the m.v.t. slowly falls to a level of about 15 $g/m^2/hr$. Thereafter the m.v.t. remains constant at this level to the end of the study.

Synthetic Dressings

When this split skin graft is covered with a synthetic dressing the water loss is immediately reduced to a mean value of 15 $g/m^2/hr$. This is still higher than water loss from normal, shaved skin, which in this experiment is approximately 7 $g/m^2/hr$. When the synthetic dressing is removed, there is a surge in moisture vapour transmission to over 100 $g/m^2/hr$. Within 30 minutes the m.v.t. was falling to control group values. A similar surge took place when the dressing was removed from all the synthetically dressed skin grafts in all groups. The peaks were similar throughout indicating that the effect of the dressing on wound humidity was maximal at one week.

Leaving the skin graft covered with the dressing after one week did not result in an increase in wound humidity.

Conclusion

The conclusion from this part of the study seems to be that a synthetic dressing allows a split skin graft to revascularise in an extremely moist environment. When this dressing is removed there is a surge in water loss which returns to the values of the control skin grafts within one hour. Thereafter the skin graft looses water at a rate which is similar to the control skin grafts. How this affects the split skin graft's behaviour will be demonstrated in the subsequent experiments.

4.4 Surface Area

Pilot Study

This study was undertaken to familiarise the author with the techniques involved in the accurate tracing of shapes with an electronic mouse. The 2x2 cm. perspex block was traced with a pen on to an acetate sheet. This was repeated 50 times. The perspex block was then photographed with the video camera and the image portrayed on the monitor was traced with the electronic mouse. The areas were then calculated by the computer.

The acetate tracings were then photographed with the same apparatus. The images produced were then traced with the mouse and the areas calculated.

The results showed that transferring the acetate tracing of the shape to the computer with the electronic mouse produced a mean surface area of 372 mm2 where as tracing the actual shape with the electronic mouse produced a mean surface area of 396 mm2. The error when tracing took place was therefore 6%.

Surface Area - Flank

All the groups studied had at least 6 rats per group. This meant that the data from each group could be analysed and compared with data from other groups.

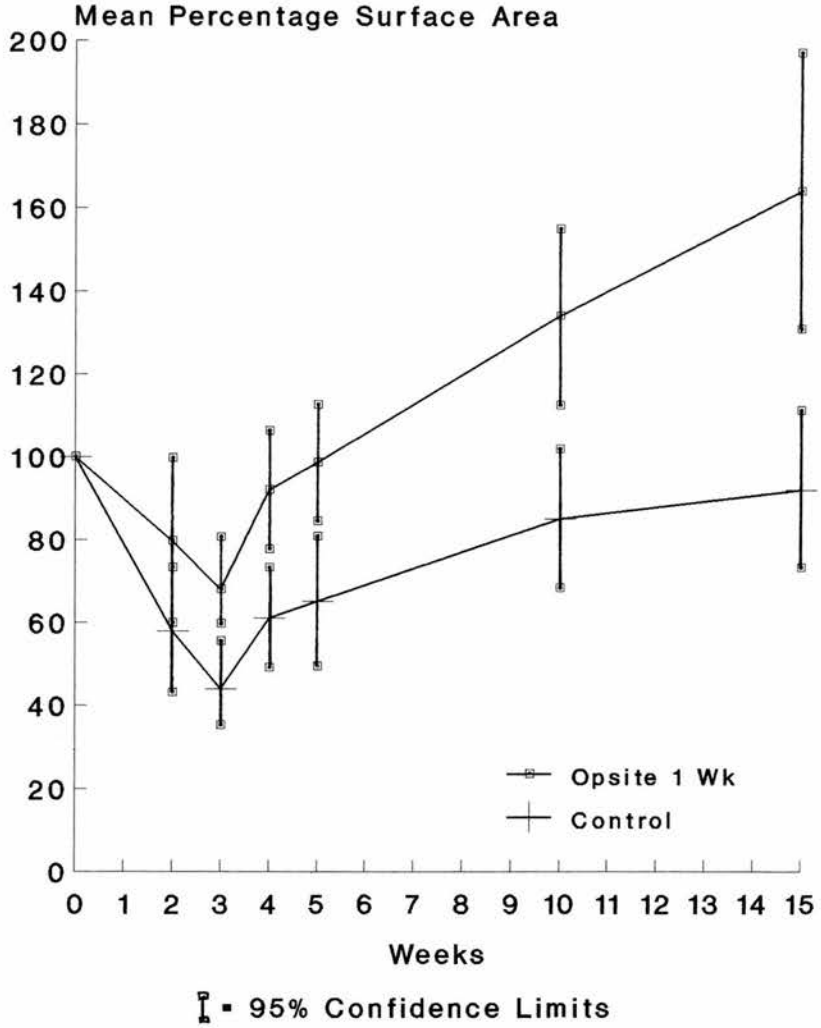
Tracings were made of all split skin grafts when they were initially applied to the open wound at operation. This initial figure would be considered the baseline and allocated a value of 100%. The second tracing was made when the animal was sacrificed. This value was then expressed as a percentage of the first figure. This would

standardise the results and allow the easier comparison of change in surface area of skin grafts. All the sub group results of one main group were then collected and graphically presented. Group A therefore consisted of data from sub groups A1,A2,A3,A4,A5,A10,A15. Group B consisted of sub groups B2,B3,B4,B5,B10,B15. Groups C, D and E were similarly constructed.

The individual sub group data is collected in tabular form in appendix 3.

Mean group surface area values with 95% confidence limits are presented in the following graphs.

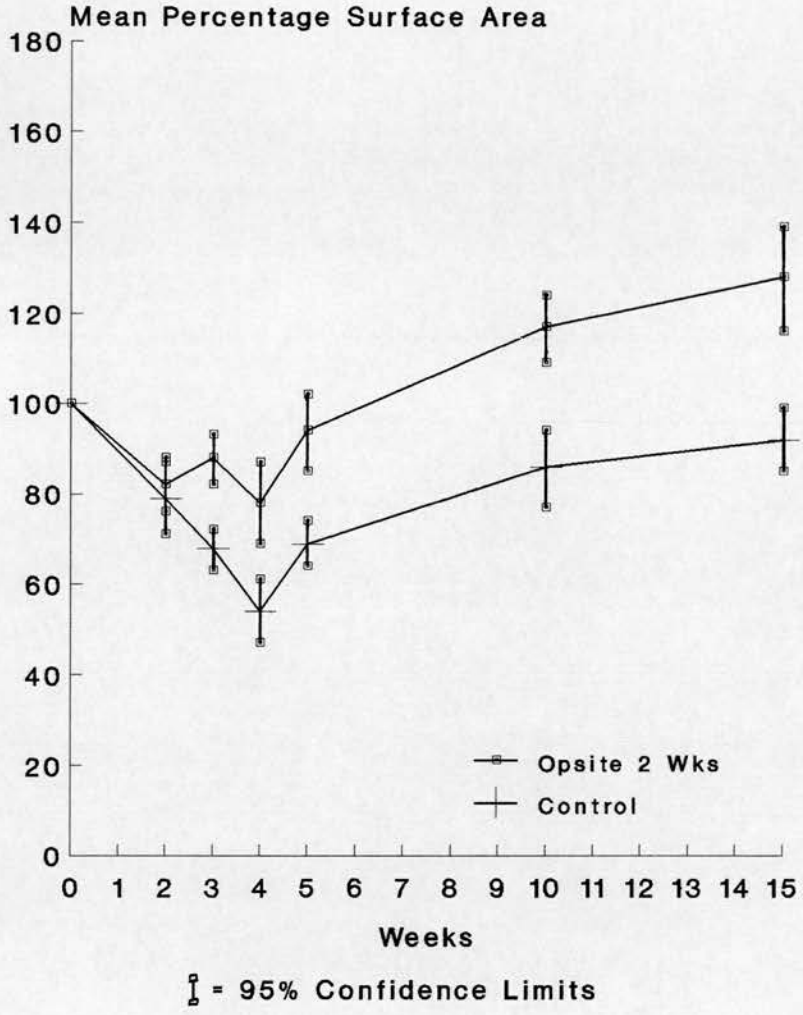
Group A Flank



Student t test comparing Opsite with Controls

	Degrees of Freedom	t	p	Mean Difference	confidence interval	
A2	6	1.494	0.1858	20.64	-6.51	49.80
A3	7	2.531	0.0390	20.61	5.18	36.04
A4	7	4.035	0.0050	30.85	16.37	45.33
A5	5	3.916	0.0112	33.52	16.27	50.76
A10	7	5.621	0.0008	48.54	32.18	64.90
A15	6	3.775	0.0094	71.66	34.58	108.74

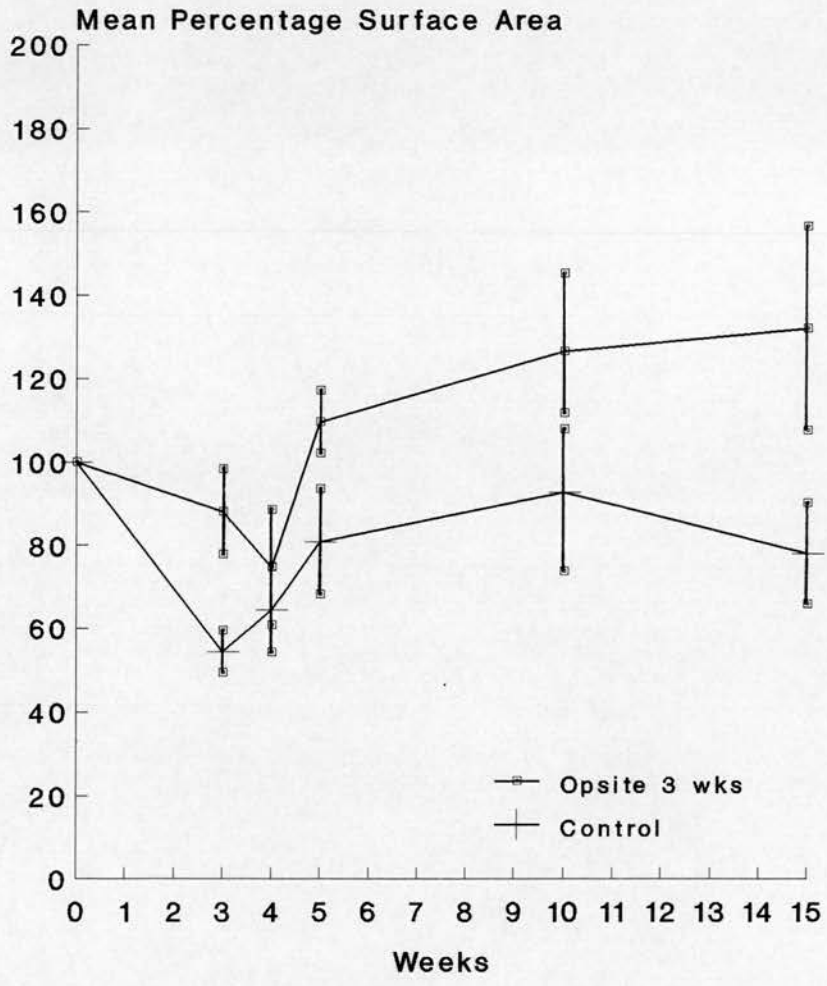
Group B Flank



Student t test comparing Opsite with Controls

	Degrees of Freedom	t	p	Mean Difference	Confidence Interval	
B2	6	0.332	0.7510	2.73	-13.23	18.69
B3	5	4.261	0.0084	20.39	10.64	30.13
B4	6	3.213	0.0183	24.36	9.63	39.09
B5	6	2.871	0.0284	24.87	8.04	41.73
B10	6	4.534	0.0040	31.27	17.87	44.67
B15	6	3.686	0.0103	36.87	17.43	56.31

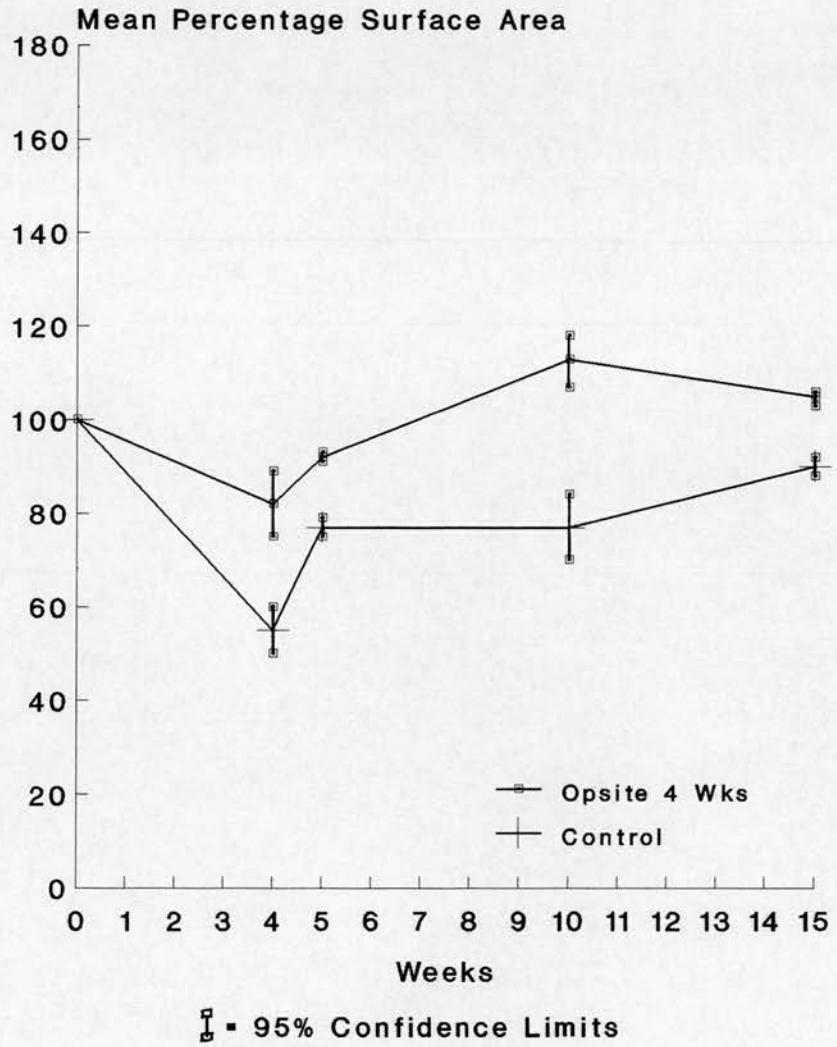
Group C Flank



Student t test comparing Opsite with Controls

	Degrees of Freedom	t	p	Mean Difference	Confidence Interval	
C3	6	8.549	0.0001	33.74	26.07	41.41
C4	7	1.402	0.2037	10.11	-3.55	23.78
C5	6	5.552	0.0014	28.71	18.66	38.76
C10	6	2.167	0.0734	33.76	3.48	64.03
C15	5	5.569	0.0026	54.03	34.48	73.58

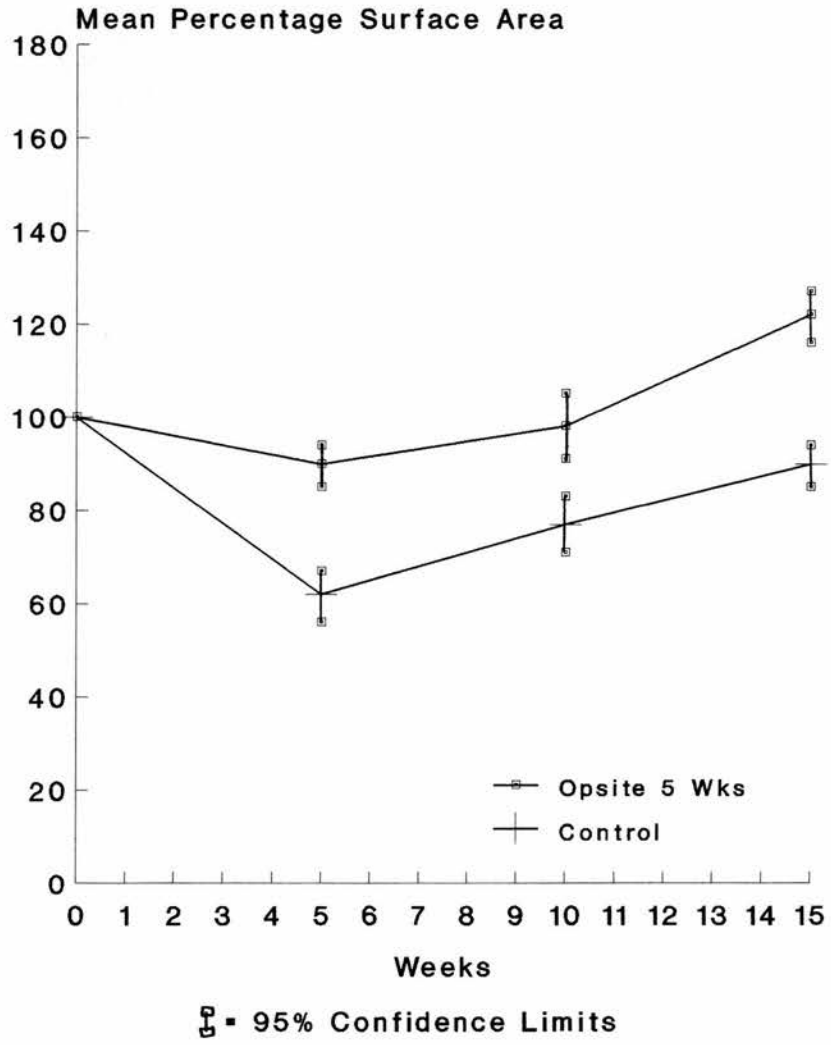
Group D Flank



Student t test comparing Opsite with Controls

	Degrees of Freedom	t	p	Mean Difference	Confidence Interval	
D4	7	2.661	0.0234	26.77	7.71	45.84
D5	7	9.561	0.0000	15.15	12.15	18.15
D10	7	7.111	0.0002	35.97	26.39	45.56
D15	6	5.269	0.0019	16.14	10.19	22.10

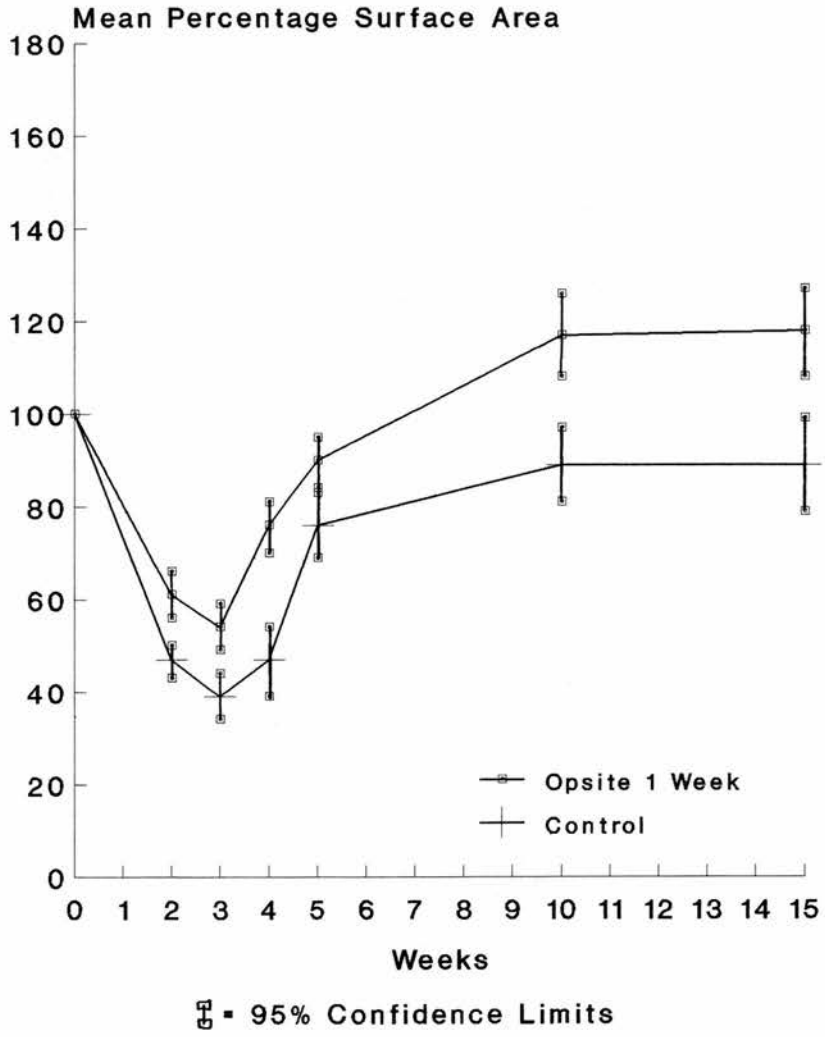
Group E Flank



Student t test comparing Opsite with Controls

	Degrees of Freedom	t	p	Mean Difference	Confidence Interval	
E5	6	3.474	0.0132	28.23	12.44	44.02
E10	6	4.651	0.0035	21.34	12.43	30.26
E15	7	4.629	0.0024	31.50	18.61	44.39

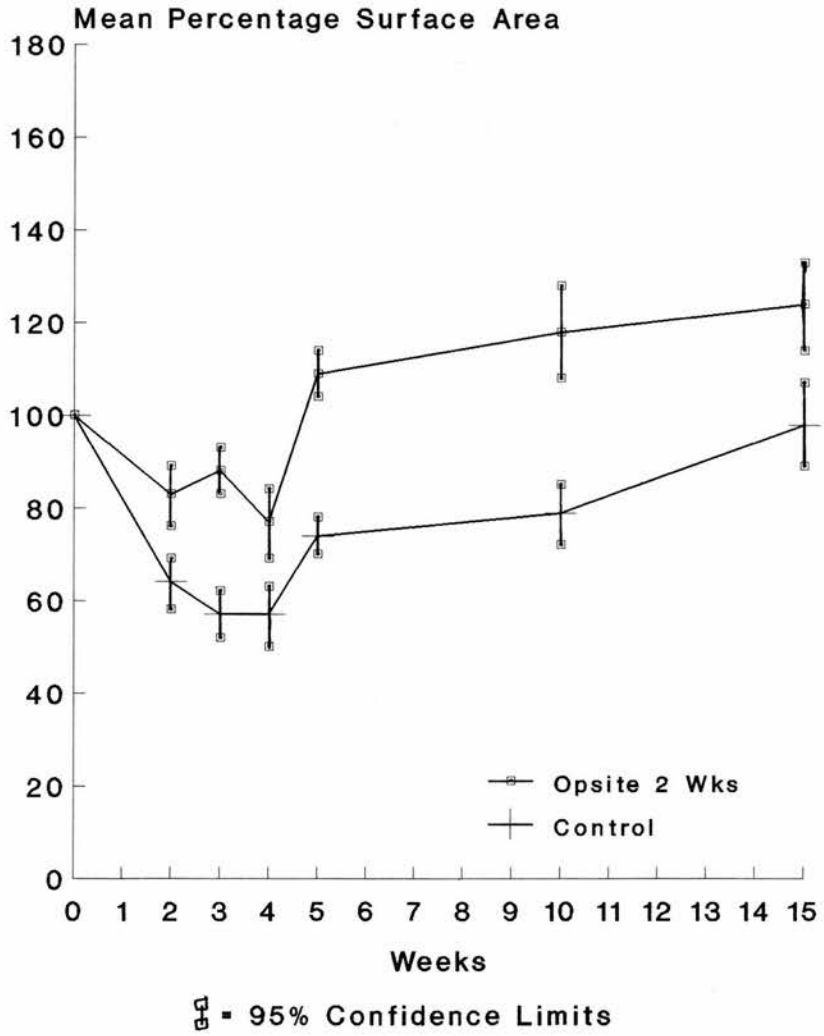
Group A Hip



Student t test comparing Opsite with Controls

	Degrees of Freedom	t	p	Mean Difference	Confidence Interval	
A2	6	1.894	0.1070	14.74	-0.38	29.87
A3	7	3.758	0.0071	14.91	7.39	22.43
A4	7	4.608	0.0025	29.46	17.35	41.58
A5	5	4.145	0.0089	13.48	6.94	20.02
A10	7	2.791	0.0269	28.29	9.08	47.49
A15	5	2.002	0.1017	30.28	-0.20	60.77

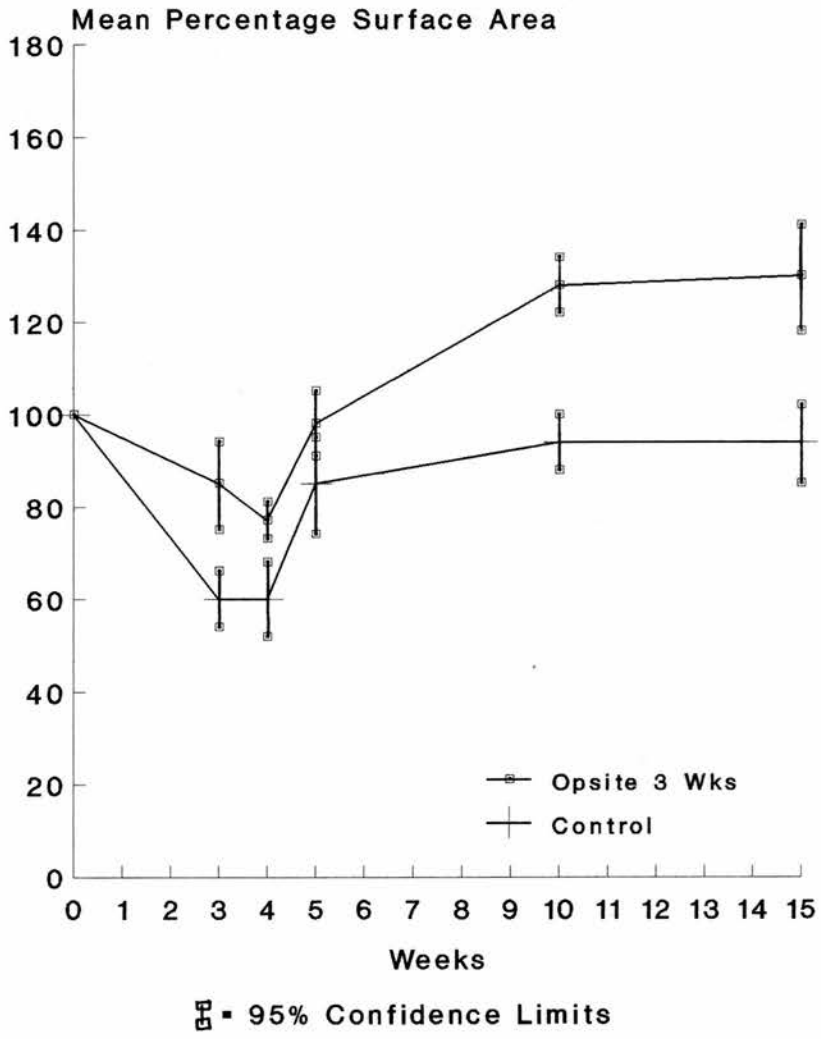
Group B Hip



Student t test comparing Opsite with Controls

	Degrees of Freedom	t	p	Mean Difference	Confidence Interval	
B2	7	2.041	0.0806	18.96	1.36	36.56
B3	5	6.099	0.0017	30.97	20.74	41.20
B4	6	4.335	0.0048	20.01	11.08	28.94
B5	5	5.930	0.0019	34.98	23.10	46.87
B10	6	5.806	0.0011	39.59	26.34	52.83
B15	6	2.091	0.8150	26.03	1.84	50.22

Group C Hip

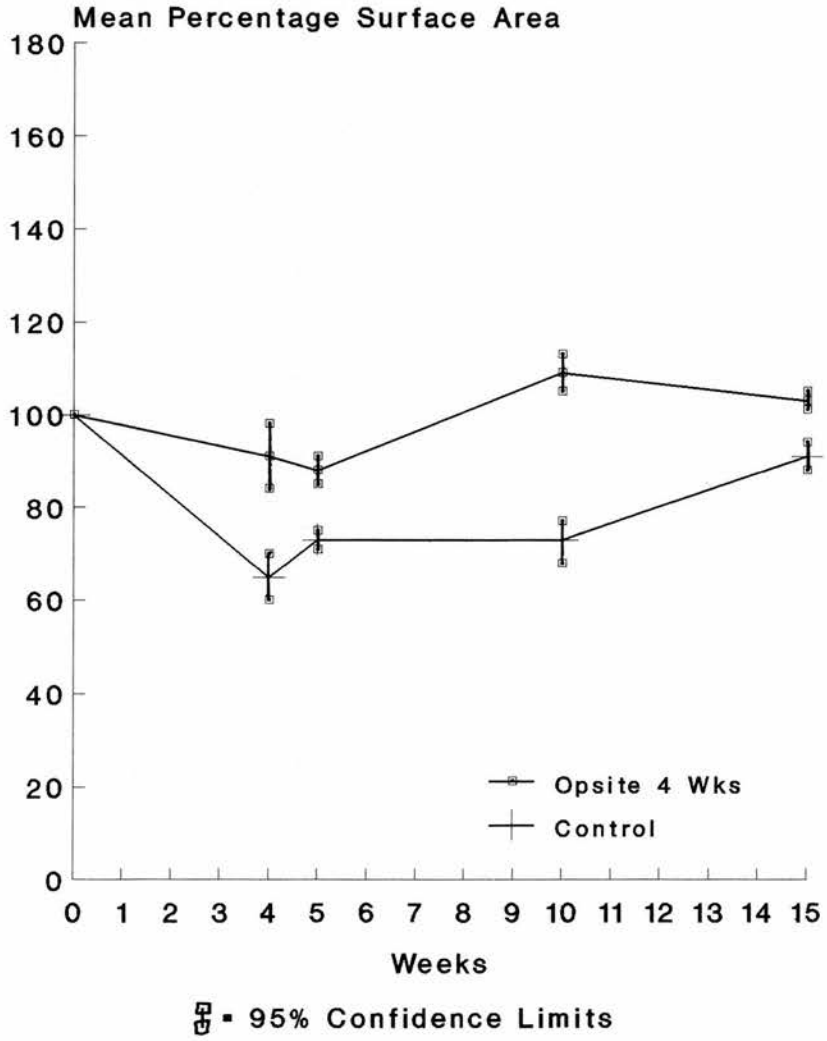


▪ 95% Confidence Limits

Student t test comparing Opsite with Controls

	Degrees of Freedom	t	p	Mean Difference	Confidence Interval	
C3	5	2.784	0.0387	24.75	6.83	42.67
C4	7	2.727	0.0295	17.22	5.26	29.19
C5	6	1.639	0.1523	12.57	-2.33	27.47
C10	6	4.337	0.0047	33.51	18.64	48.39
C15	5	3.191	0.0242	35.82	13.20	58.44

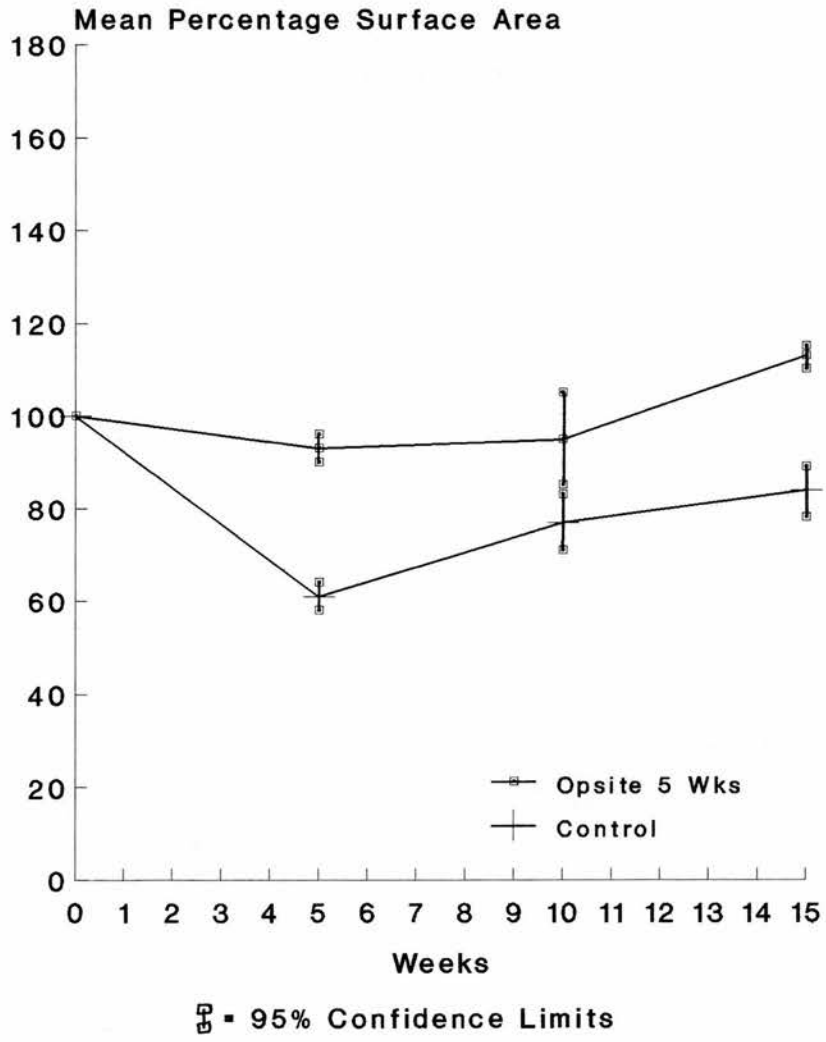
Group D Hip



Student t test comparing Opsite with Controls

	Degrees of Freedom	t	p	Mean Difference	Confidence Interval	
D4	7	3.258	0.0139	26.31	11.01	41.62
D5	7	5.608	0.0008	14.50	9.60	19.40
D10	7	11.387	0.0000	35.25	29.38	41.12
D15	6	2.898	0.0274	11.97	3.95	20.00

Group E Hip



Student t test comparing Opsite with Controls

	Degrees of Freedom	t	p	Mean Difference	Confidence Interval	
E5	6	5.919	0.0010	31.81	21.37	42.26
E10	6	2.410	0.0526	17.69	3.43	31.94
E15	7	4.411	0.0031	29.46	16.18	42.12

Summary

Surface Areas Flank

Control Grafts

During the first 3-4 weeks graft size fell to a mean of 56% its original size. This sharp fall in surface area is not unique to this study. Although it is not commented upon, this phenomenon occurs in published data on skin graft contraction
(Rudolph 1979, Corps 1969). If one compares the weights of the rats during this time in this study, then it may be that the weight loss during this time has influenced graft size as surface area is related to body weight (see page 118). This will be evaluated later.
After 4 weeks the skin grafts then went on to increase in size and at the end of the study the grafts had reached 80-90% their original size. These figures are similar to published animal work (Corps 1969, Rudolph 1979) and compares with the clinical behaviour of human split skin grafts (Padgett 1942, Grabb and Smith 1979).

Skin Grafts Synthetically Dressed

The split skin grafts covered with a synthetic dressing behaved in a manner similar to the control skin grafts for the first 3-4 weeks following wounding. After this time the mean size of all the skin grafts synthetically dressed increased at a rate greater than the control grafts.

Group A

The skin grafts in this group were covered with a synthetic dressing for one week. The wounds shrank to a mean of 68% their original size at 3 weeks. The wounds

then increased in size until at 15 weeks they were 164% their original size.

Groups B,C,D,and E.

The skin grafted wounds covered with the synthetic dressing in these groups underwent an initial reduction in size similar to the control grafts. However, the contraction was always less in the synthetically dressed group. The skin grafts in these groups then went on to increase in size and at 15 weeks post wounding they were all larger that their respective (in-animal) controls. The optimum time for covering a skin graft with a synthetic dressing was one week, grafts covered for longer periods received no added benefit.

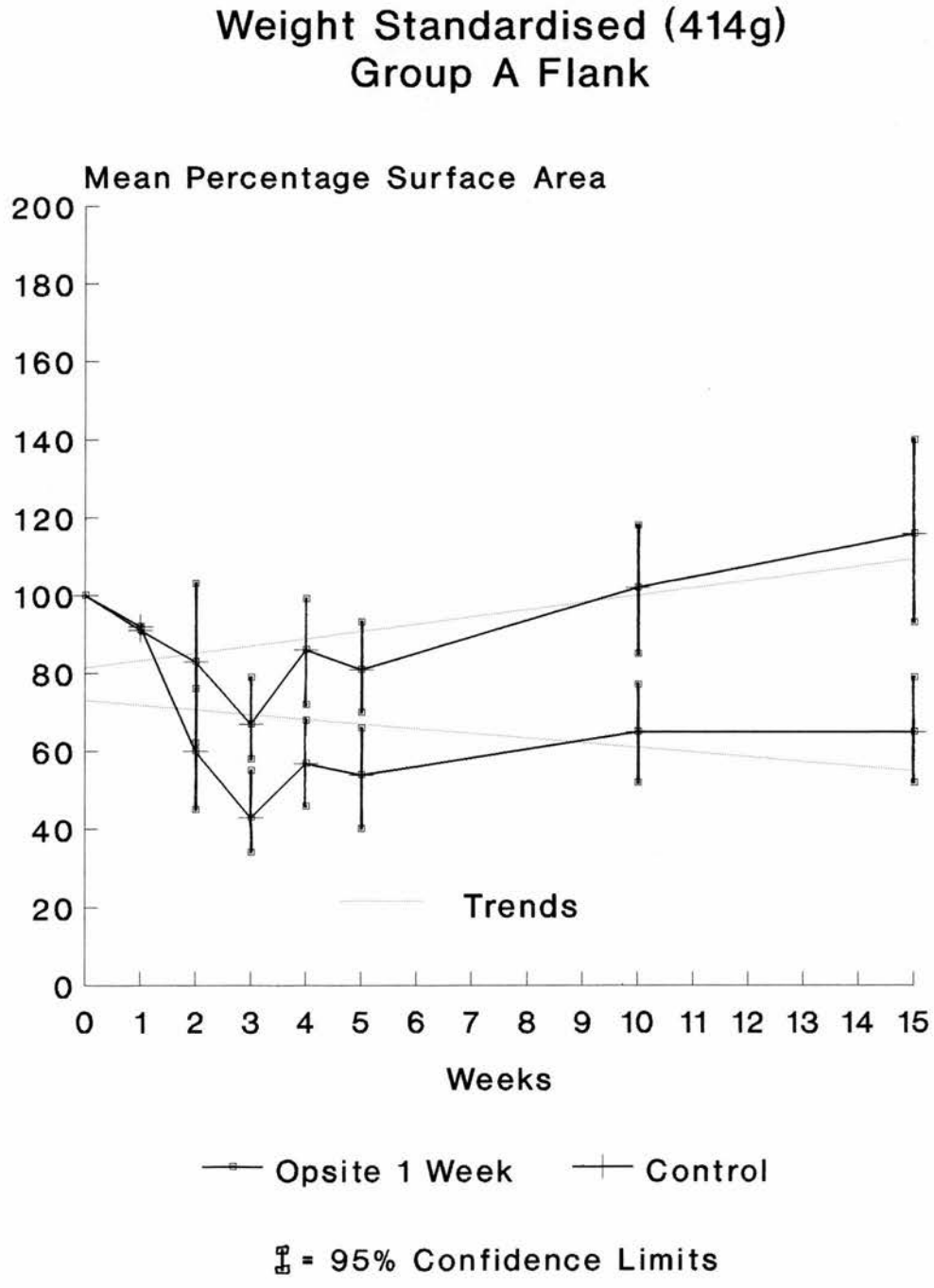
Surface Areas Hip

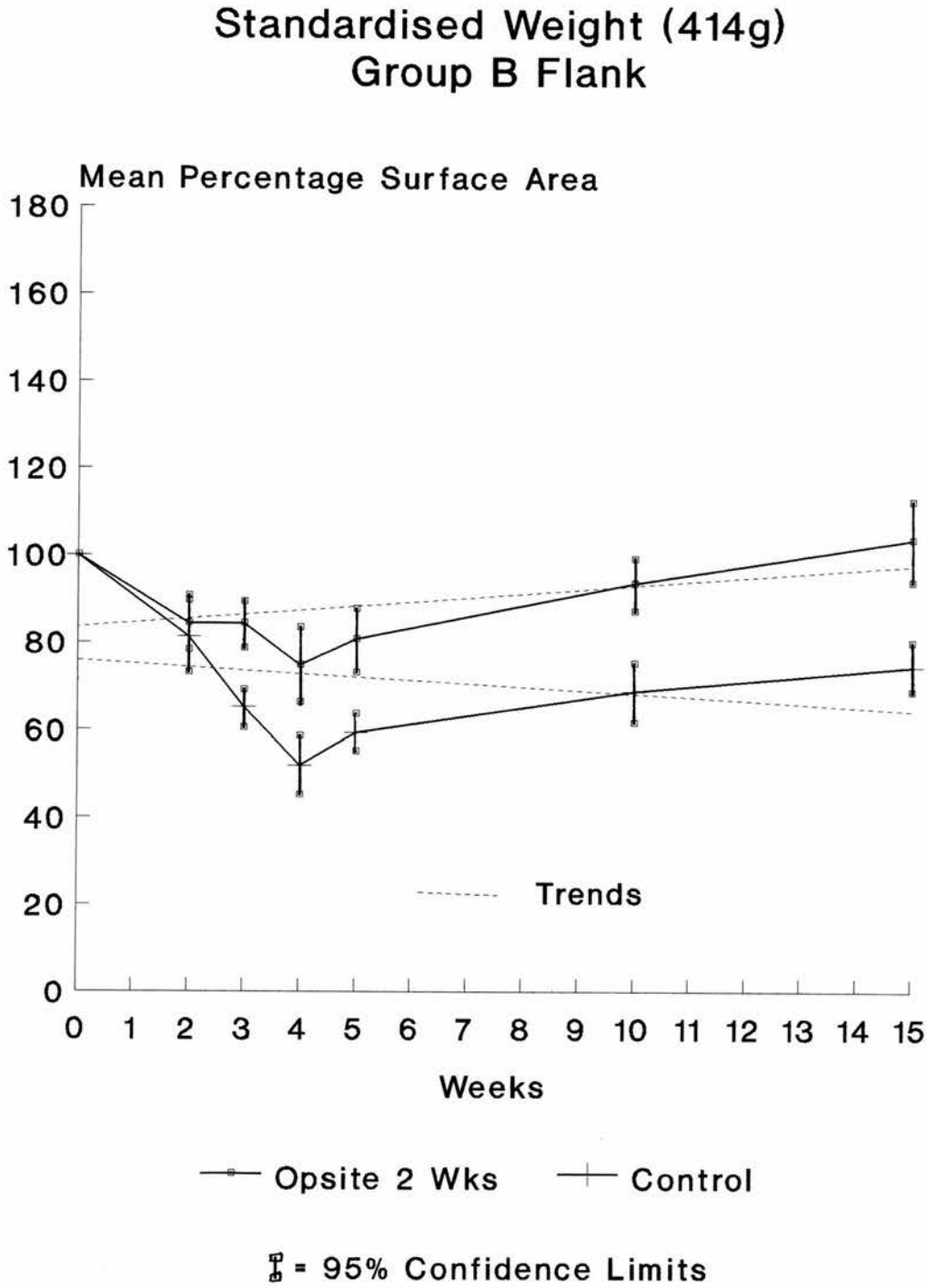
It is interesting that all grafts in this area undergo contraction in the first 3-4 weeks following surgery. This response is similar to the skin grafted wounds on a non-flexor surface (flank area). After 4 weeks there is an increase in size of all grafts; the size of the synthetically dressed grafts being larger than the in animal controls.

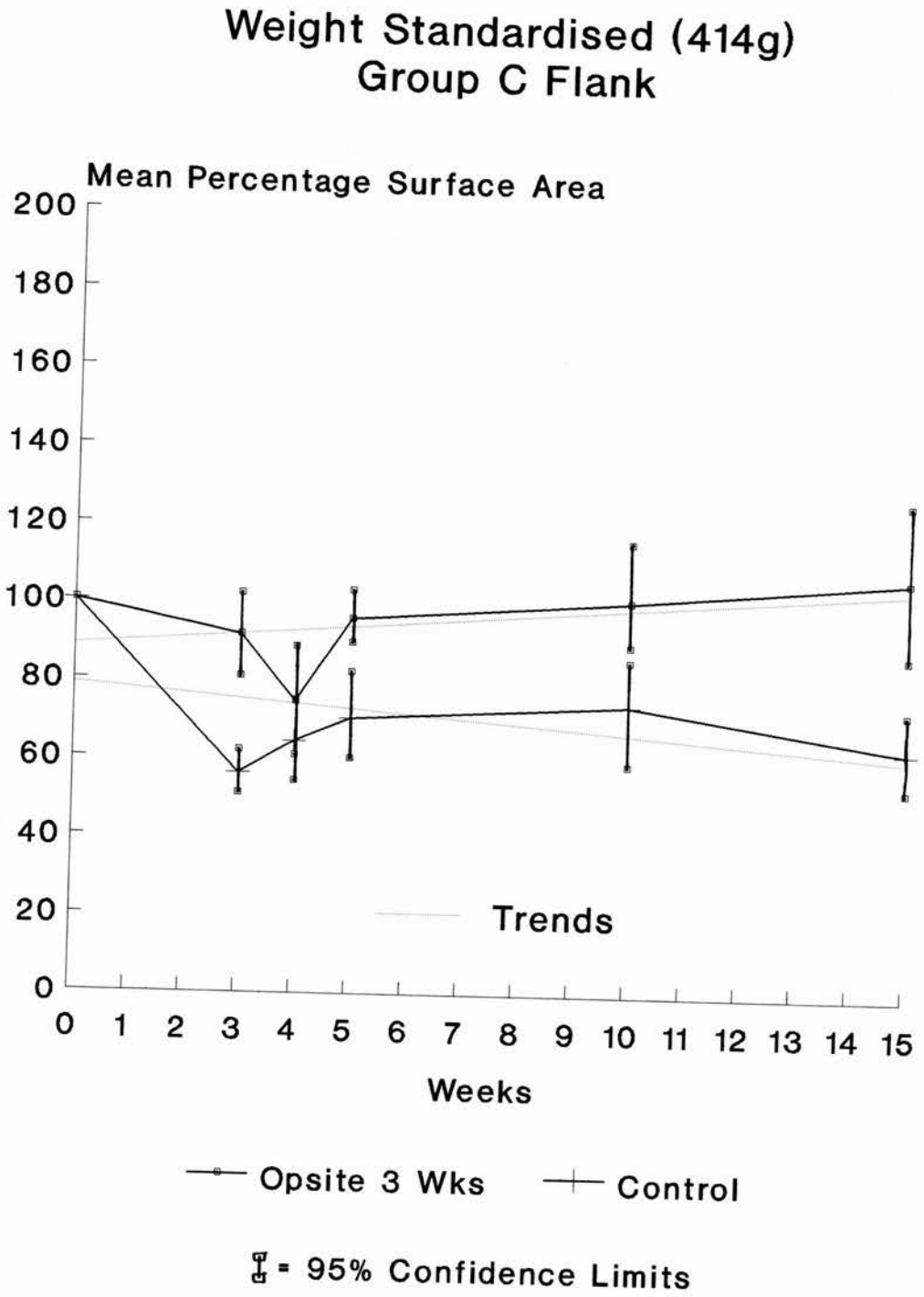
Discussion

The control skin grafts in this study contracted in a manner similar to published data by never regaining their original size (Rudolph 1979, Corps 1969, Padgett 1952). Surface area is directly related to animal weight. Surface area estimation was carried out on individual sub groups at various points in the study when animal weight varied. If mean group weight therefore is standardised to 414 grams the following charts allow direct comparison of

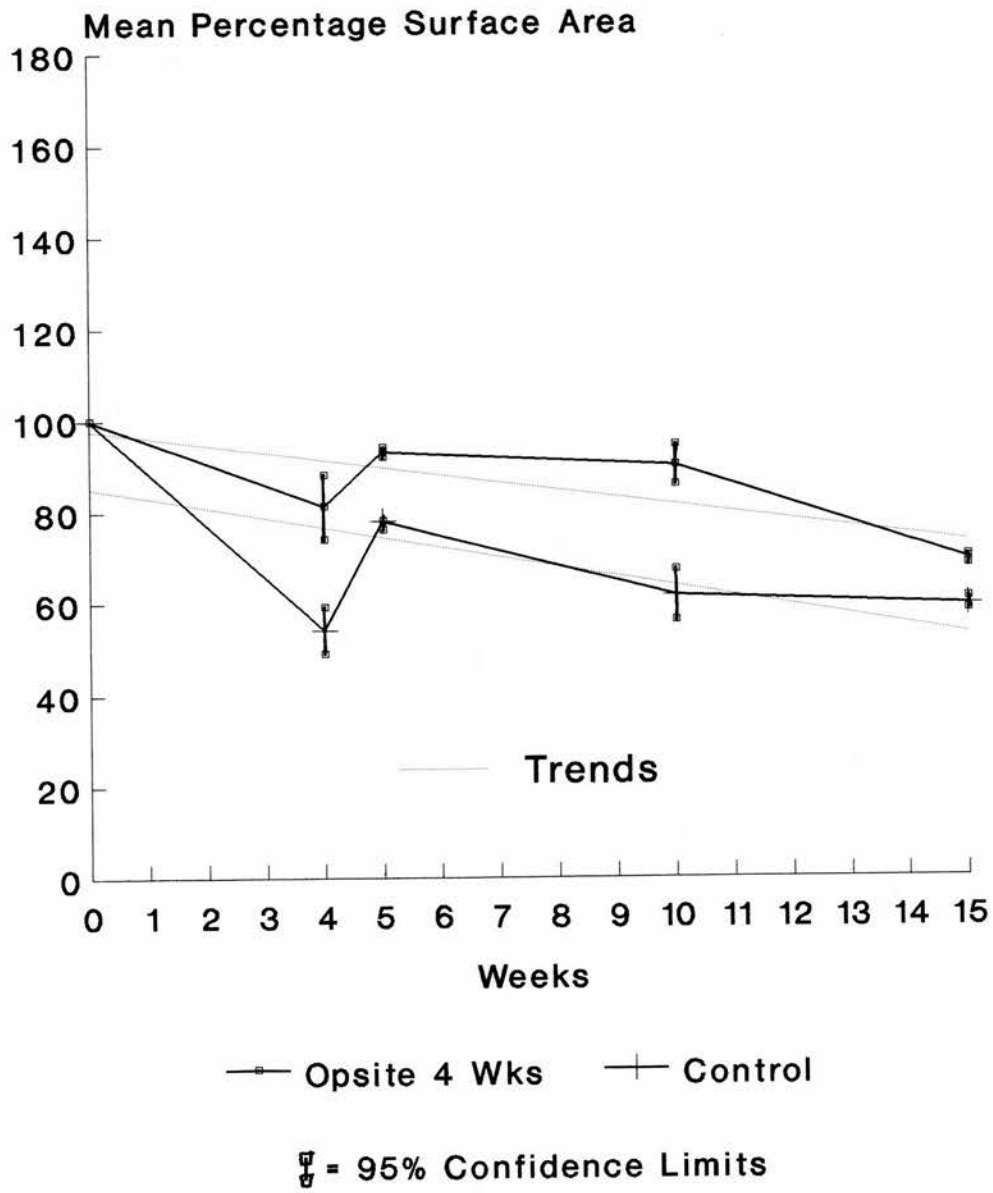
surface areas within groups and between groups.

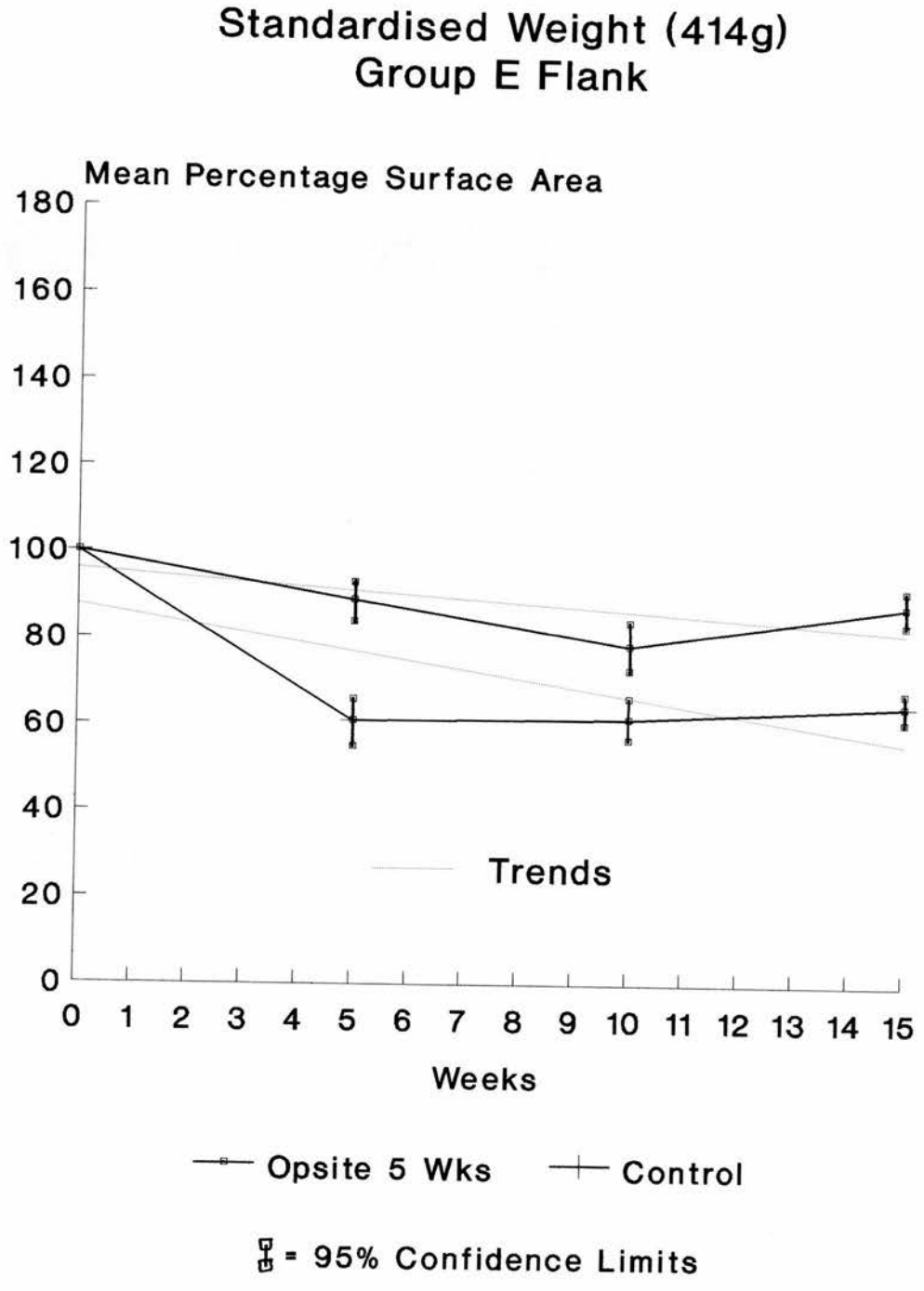



Standardised Weight (414g)
Group B Flank
Mean Percentage Surface Area
180
160
140
120
100
80
60
40
20
0
Trends
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Weeks
Opsite 2 Wks
Control
= 95% Confidence Limits


Weight Standardised (414g)
Group C Flank
Mean Percentage Surface Area
200
180
160
140
120
100
80
60
40
20
0
Trends
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Weeks
Opsite 3 Wks
Control
= 95% Confidence Limits

Standardised Weight (414g)
Group D Flank




Standardised Weight (414g)
Group E Flank
Mean Percentage Surface Area
180
160
140
120
100
80
60
40
20
0
Trends
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Weeks
Opsite 5 Wks
Control
= 95% Confidence Limits

Standardising rat weight reduces the magnitude of the values but because in-animal controls were used the difference between the control grafts and the opsite covered grafts is similar.

Trends

Controls

The trend of the control grafted wounds was negative, indicating the propensity of these grafts to reduce in size. The slope of all the control graphs was similar indicating their homogeneity.

Synthetic Dressings

All grafts synthetically dressed in groups A,B and C had a positive or neutral trend. The grafts in these groups therefore had a tendency to either increase in size in keeping with their weight gain or to increase in size at a rate greater than their weight gain. Grafts in groups D and E had a negative trend. These grafts therefore did not increase in size at the same rate as the rat gained weight. Although the slope was less steep in the grafts synthetically dressed compared to their control grafts, it would appear that if the synthetic dressing remains in situ for periods longer than three weeks the beneficial effect on surface area is reduced.

4.5 Myofibroblast Cell Population

These modified fibroblasts are difficult to assess individually as they form clumps and each cell becomes intimately associated with its neighbours. Previous studies have identified myofibroblasts using the electron microscope, Rudolph (1979) counted fibroblasts with a "myofibroblast appearance". It is therefore accepted that the assessment of cell numbers in this situation cannot be 100% accurate whichever method is used to first of all identify then secondly to count these cells. Using an immunoperoxidase stain linked to a specific monoclonal antibody targeted to the intracellular actin moiety of the myofibroblast would increase the accuracy and ease of identification of these cells. Even this method is not 100% accurate. McGrath (1982) used this method when identifying myofibroblasts with sequential slices of tissue. She built up 2 dimentional maps of cell distribution and statistically analysed this data. However, on examination of the photomicrographs in this paper, it would seem to be very difficult to identify individual cells and it is possible that various sections of one cell were counted as several cells. Photomicrographs (overleaf) of the myofibroblasts identified in this present study indicate the difficulty in individual cell identification.

The photograph below shows a low powered view of one section of a skin grafted wound. Clumps of myofibroblasts (stained brown) can be identified at the skin graft - wound interface.

This high powered view gives an indication of the degree of difficulty in individual cell identification. This * would be regarded as one cell clump for simplicity.

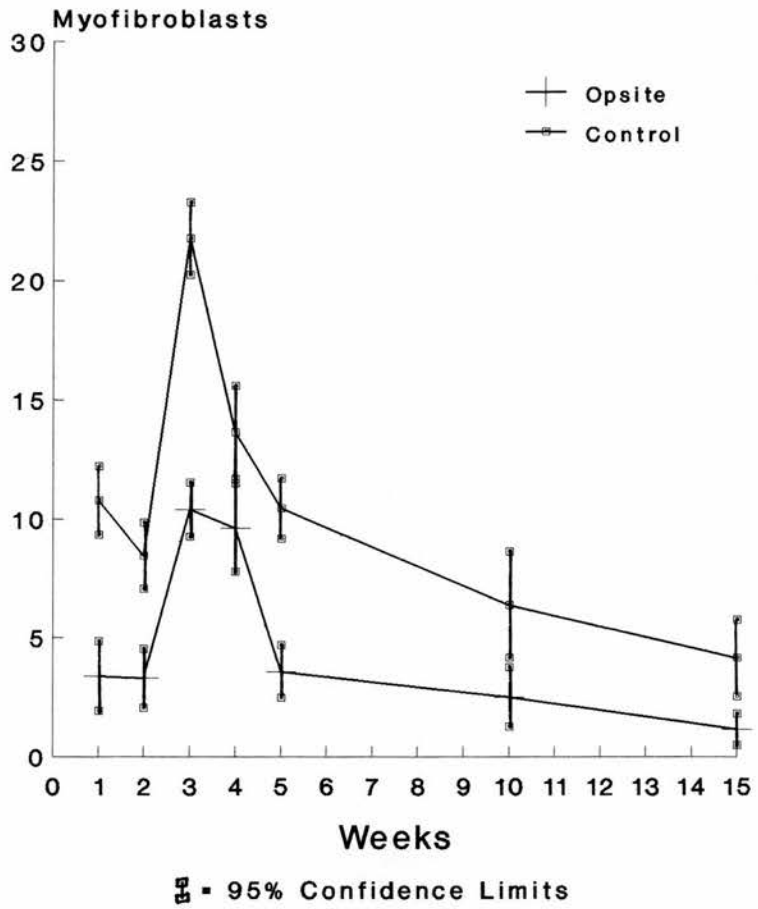
This would then mean that the main innacuracy of this method would be in the supposition that each clump contained exactly the same number of cells. The likelyhood of this being the case is remote but it is assumed that the innacuracy of this method is the same throughout the experiment thus allowing comparison within the study. This method may be no more inaccurate than the time consuming methods previously described.

The aim of the study was to compare the differences between wounds covered with different dressings. Therefore although data from this study could not be compared with data from other studies an indication of the difference induced in myofibroblast numbers by the different dressings could be concluded.

Myofibroblast Cell Numbers - Flank

The following graphs represent the numbers of myofibroblast cell clumps per high powered field per unit surface area in each main group.

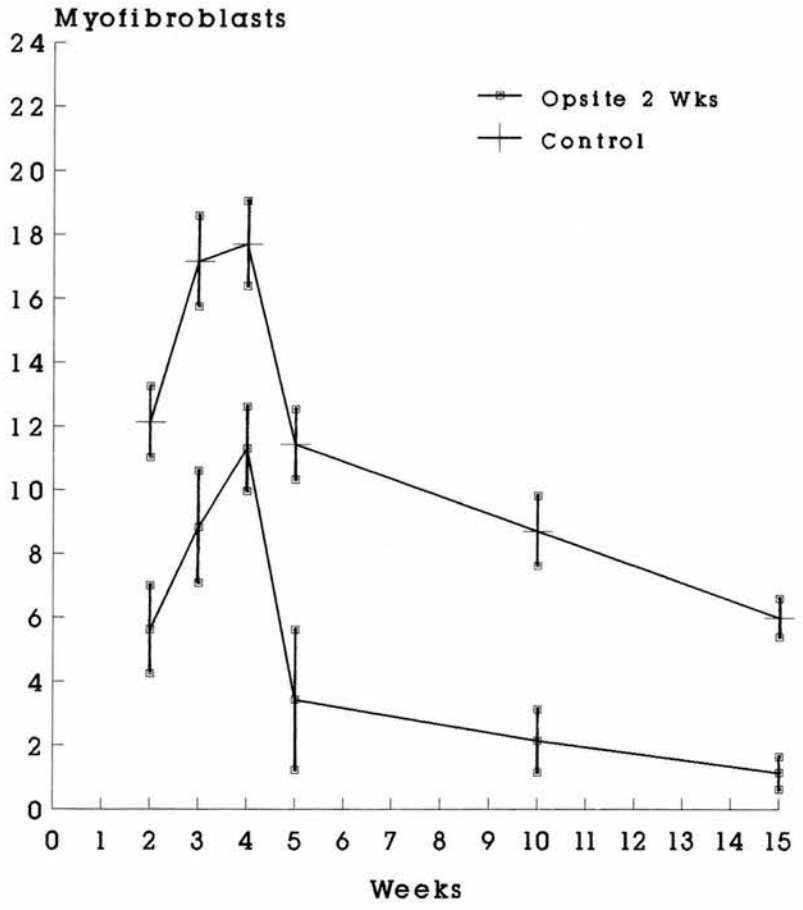
Group A
Flank



Student t test comparing Opsite with Controls

Weeks	1	2	3	4	5	10	15
Freedom	7	6	7	7	6	7	6
"t"	6.099	4.500	9.524	2.244	6.429	4.329	3.674
"p"	0.0005	0.0041	0.0000	0.0598	0.0006	0.0034	0.0104
Mean Diff.	7.37	5.14	11.37	4.00	6.86	3.88	3.00
95% Conf. Limits	4.52	2.35	8.55	-0.22	4.27	1.76	1.00
	10.23	7.94	14.20	8.22	9.44	5.99	5.00

Group B
Flank

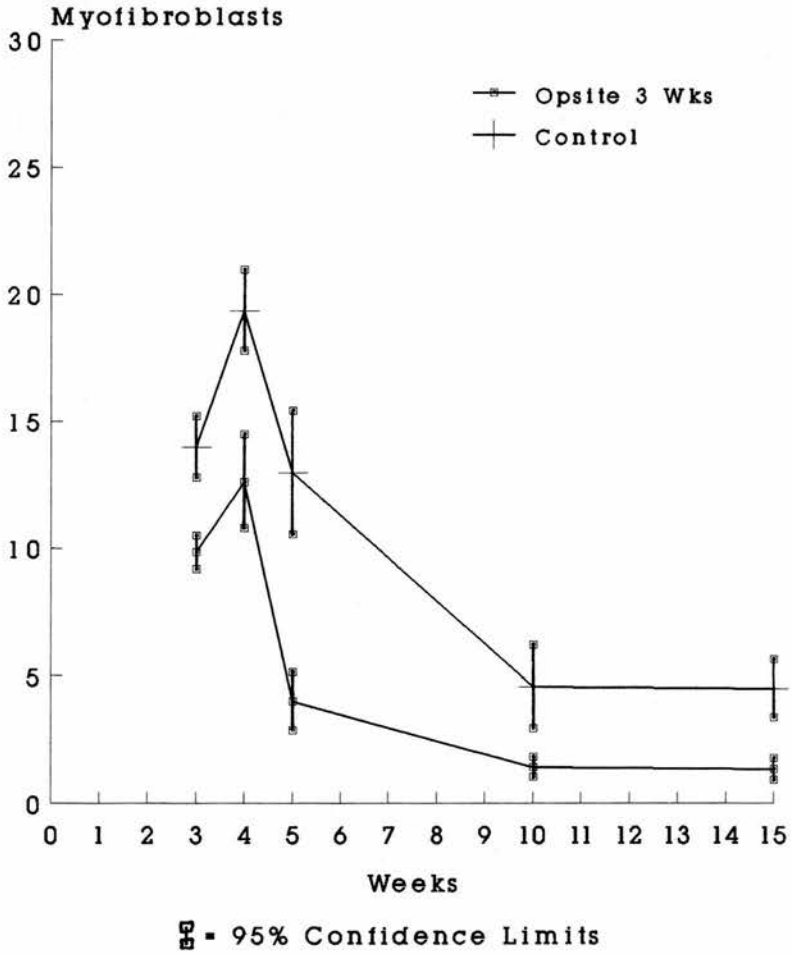


= 95% Confidence Limits

Student t test comparing Opsite with Controls

Weeks	2	3	4	5	10	15
Freedom	7	5	6	6	6	6
"t"	6.619	10.381	5.793	8.892	17.816	9.553
"p"	0.0003	0.0001	0.0012	0.0001	0.0000	0.0001
Mean Difference	6.50	8.33	6.43	8.00	6.57	4.88
95% Confidence Limits	4.18 8.82	6.27 10.40	3.71 9.14	5.80 10.20	5.67 7.47	3.61 6.10

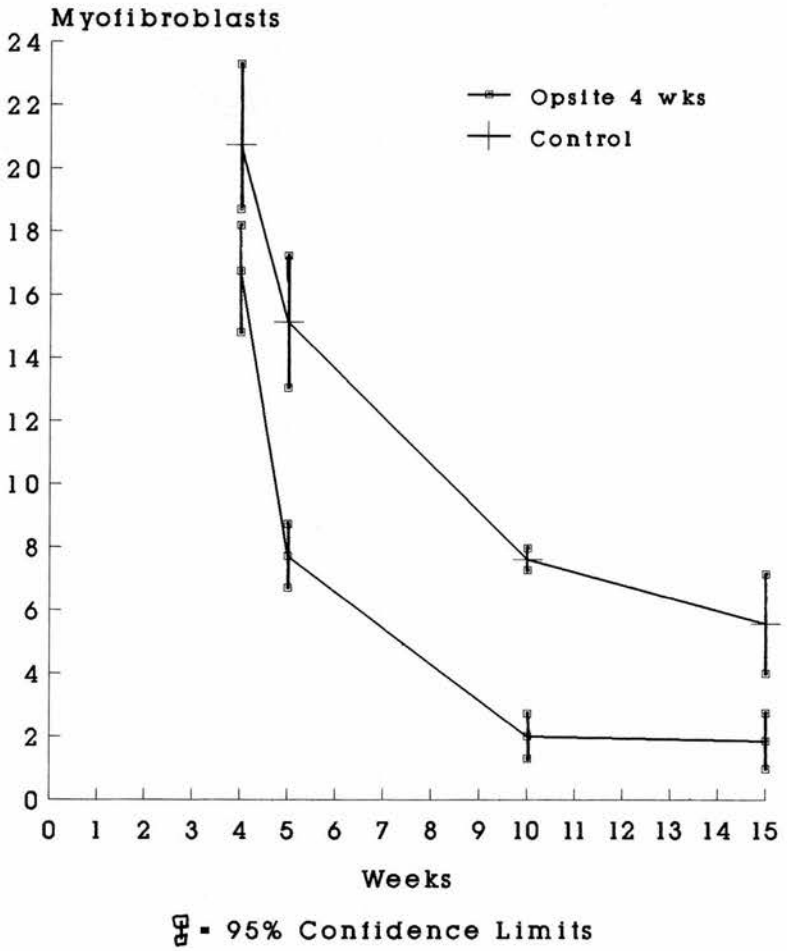
Group C
Flank



Student t test comparing Opsite with Controls

Weeks	3	4	5	10	15
^{0}Freedom	6	7	6	6	5
"t"	4.833	6.906	8.088	4.085	6.635
"p"	0.0029	0.0002	0.0002	0.0065	0.0012
Mean Difference	4.14	6.75	9.00	3.14	3.17
95% Confidence Limits	2.05 6.24	4.44 9.06	6.28 11.72	1.26 5.03	1.94 4.39

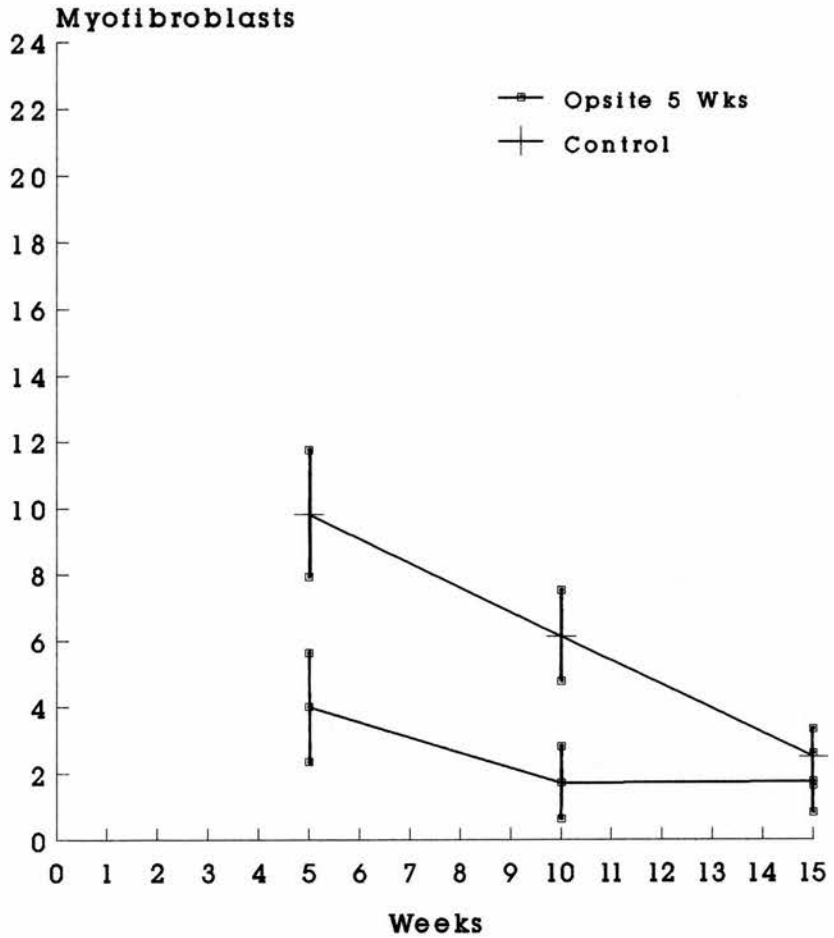
Group D
Flank



Student t test comparing Opsite with Controls

Weeks	4	5	10	15
Freedom	7	6	7	6
"t"	2.804	5.461	10.565	3.495
"p"	0.0264	0.0016	0.0000	0.0129
Mean Difference	4.00	7.43	5.63	3.71
95% Confidence Limits	0.63 7.37	4.10 10.76	4.37 6.88	1.11 6.31

Group E
Flank



I = 95% Confidence Limits

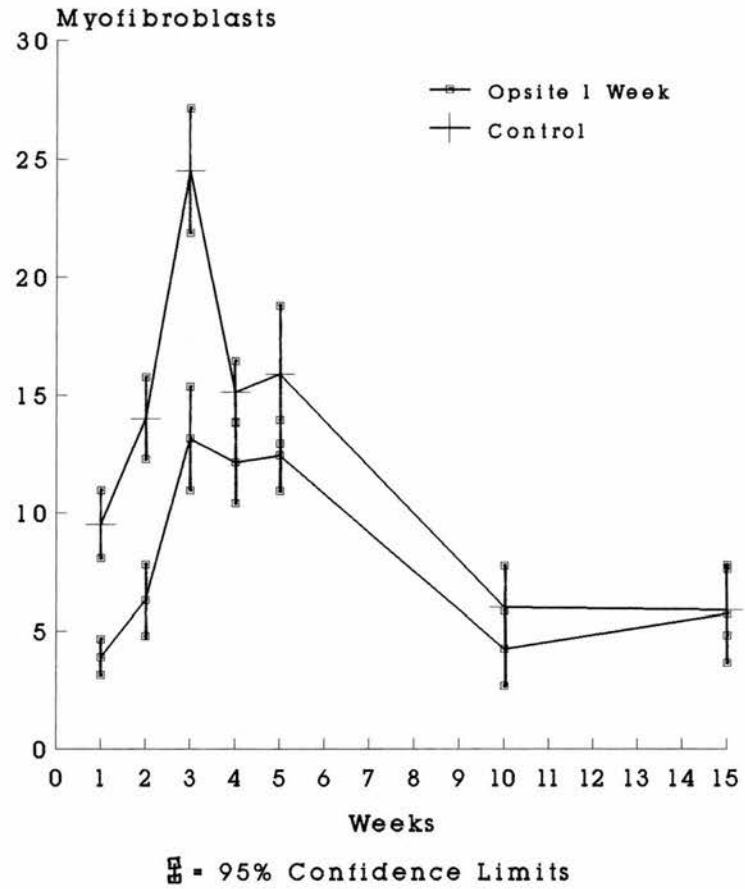
Student t test comparing Opsite with Controls

Weeks	5	10	15
Freedom	5	6	7
"t"	3.693	3.845	1.271
"p"	0.0141	0.0085	0.2443
Mean Difference	5.83	4.43	0.75
95% Confidence Limits	1.77 9.89	1.61 7.25	-0.65 2.15

Myofibroblast Cell Numbers - Hip

The numbers of myofibroblast cell clumps were identified and counted in the split skin grafts covering the hip wounds in exactly the same way as those of the flank.

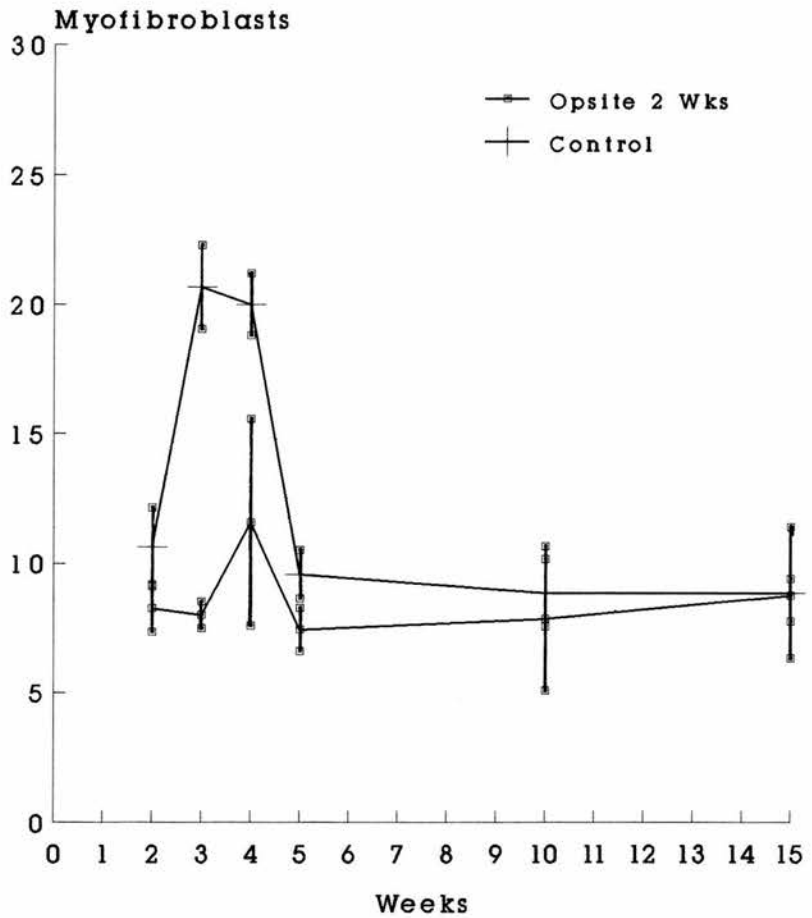
Group A
Hip



Student t test comparing Opsite with Controls

Weeks	1	2	3	4	5	10	15
^{0}Freedom	7	6	7	7	6	7	6
"t"	6.666	5.610	5.155	2.479	3.032	1.030	0.091
"p"	0.000	0.002	0.001	0.042	0.023	0.337	0.930
Mean Diff.	5.63	7.71	11.75	3.00	3.43	1.75	0.14
95% Conf. Limits	3.63 7.62	4.35 11.48	6.32 17.18	0.14 5.86	0.66 6.20	-2.27 5.77	-3.69 3.97

Group B
Hip

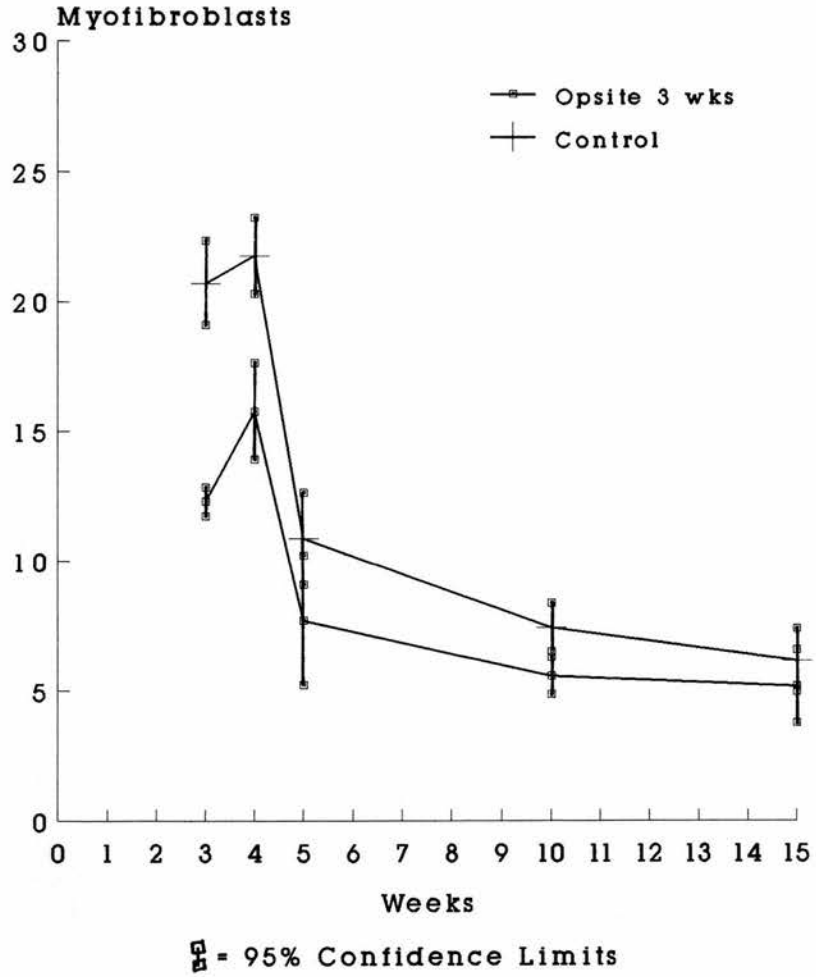


⊟ = 95% Confidence Limits

Student t test comparing Opsite with Controls

Weeks	2	3	4	5	10	15
^{0}Freedom	7	5	6	6	6	6
"t"	2.468	15.779	3.772	2.423	0.579	0.253
"p"	0.043	0.000	0.009	0.052	0.573	0.808
Mean Difference	2.38	12.67	8.43	2.14	1.00	0.29
95% Confidence Limits	0.10 4.65	10.60 14.73	2.96 13.90	-0.02 4.31	-3.10 5.10	-2.47 3.05

Group C
Hip

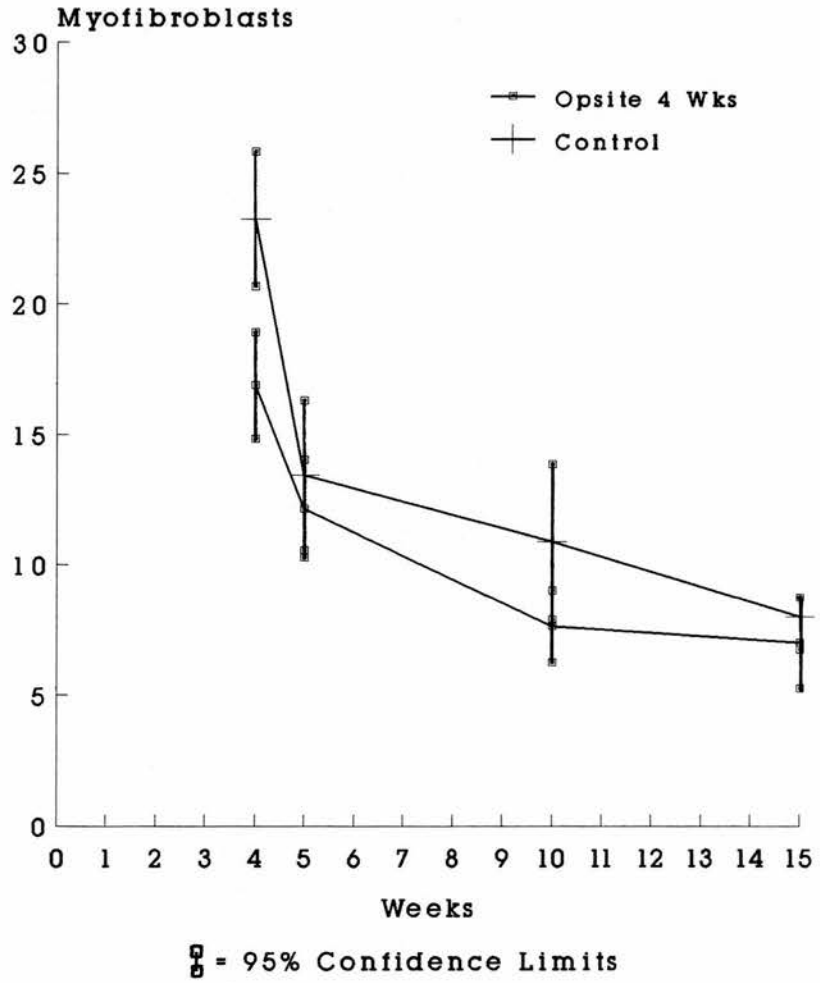


= 95% Confidence Limits

Student t test comparing Opsite with Controls

Weeks	3	4	5	10	15
Freedom	6	7	6	6	5
"t"	7.747	4.681	2.238	2.414	2.739
"p"	0.000	0.002	0.067	0.052	0.041
Mean Difference	8.43	6.00	3.14	1.86	1.00
95% Confidence Limits	5.77 11.09	2.97 9.03	-0.29 6.58	-0.03 3.74	0.06 1.94

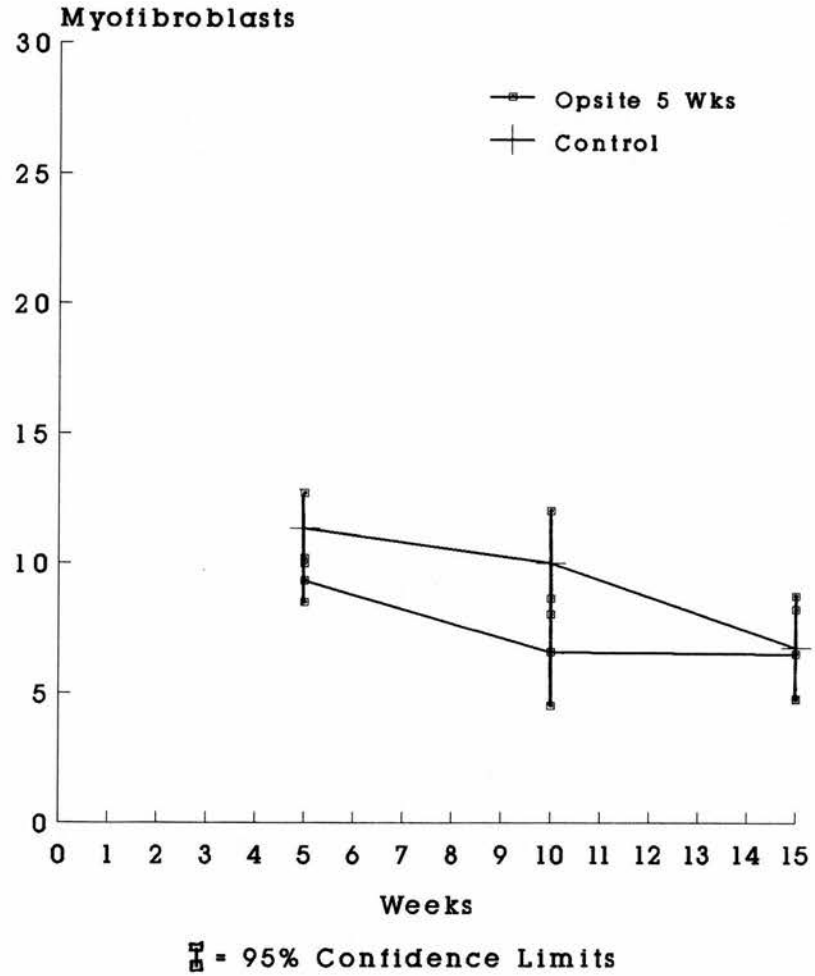
Group D
Hip



Student t test comparing Opsite with Controls

Weeks	4	5	10	15
Freedom	7	6	7	6
"t"	3.444	0.862	1.598	0.725
"p"	0.011	0.422	0.154	0.496
Mean Difference	6.38	1.29	3.25	1.00
95% Confidence Limits	2.00 10.75	-2.36 4.94	-1.56 8.06	-2.38 4.38

Group E
Hip



T = 95% Confidence Limits

Student t test comparing Opsite with Controls

Weeks	5	10	15
^{0}Freedom	5	6	7
"t"	2.236	4.221	0.323
"p"	0.076	0.006	0.756
Mean Difference	2.00	3.43	0.25
95% Confidence Limits	-0.30 4.30	1.44 5.42	-1.58 2.08

Summary

The myofibroblast numbers in each sub group were counted. The mean including 95% confidence intervals were calculated. Further evaluation was carried out using the paired student's t test.

Myofibroblasts Flank

Myofibroblast cells were always present in smaller numbers under skin grafts covered with opsite. In both the control and the synthetically dressed groups there was a peak concentration at 3-4 weeks post wounding. The peak numbers were larger in the control groups. At 5 weeks numbers of myofibroblast cell clumps fell in both groups to small but significant numbers. These numbers leveled out and at 15 weeks post skin grafting myofibroblasts were still present in both groups. The numbers in the groups where the skin grafts were synthetically dressed were smaller than the control groups.

Control Groups

The numbers in the control groups were similarly scattered. Groups A,B, and C, registered peaks of 17-20 cell clumps per high powered field per unit surface area at 3,4,and 4 weeks respectively. Thereafter myofibroblast cell numbers fell to between 6-10 cell clumps per high powered field per unit surface area at 15 weeks.

Groups Synthetically Dressed

The mean cell count peaked in all groups at 4 weeks. The cell numbers then fell to below 10 cells per high powered field at 5 weeks and then to below 5 cells per high powered field at 10 weeks.

Myofibroblast Cell Numbers Hip

The myofibroblast numbers in the hip wounds vary in a similar way to the flank myofibroblasts. The control wounds have larger cell counts and peak at 3-4 weeks. They then fall to approximately 10 cells per high powered field at 5 weeks and remain at this level to 15 weeks. The synthetically dressed wounds commence with fewer myofibroblasts per high powered field. The peak at 4 weeks in these groups is less than the corresponding control peaks, but after 5 weeks the numbers in both groups are statistically similar.

This technique only counted myofibroblasts with actin in their cytoplasm. Therefore myofibroblasts without intracellular actin would not be identified. This is advantageous as Darby (1990) states that it is only those cells with intracellular actin that actively participate in wound contraction. This method therefore gives an indication of the contraction potential within the wound. It does not give the full answer however as the method cannot quantify the amount of intracellular actin present in each cell. It can be concluded that the numbers of myofibroblasts identified give an indication of the potential for wound contraction - the extent of such contraction must be determined by other (pathophysiological) means.

Human Study

This investigation was commenced to see if the occlusive dressing that was used in the preceding animal experiments had a similar effect on wound contraction in human wounds.

5.1 Experimental Design

This study was designed to investigate the effect of an occlusive dressing on the contraction of wounds covered with a split skin graft.

Twenty two patients with bilateral forearm tattoos were entered into the study. The tattoos were of such a size that, following their excision, a split skin graft would be required to cover the defect.

Procedure

Under general anaesthesia the tattoo on each forearm was removed with all aspects of the reticular dermis. This left a wound with no macroscopic dermal contents. When bleeding took place from the subcutaneous fat a sterile paper towel was applied and held tightly to the wound for a few seconds, the shape made was then carefully cut out and applied to the wound to ensure that the template was an accurate copy of the wound. This template was then traced onto an sheet of paper and carefully stored for later surface area measurement.

A split skin graft was then taken with a powered dermatome from the inner aspect of the thigh. The dermatome was set to harvest a split skin graft of similar thickness throughout. The size of the split skin graft harvested was intentionally made large enough to cover both wounds and leave enough excess skin to allow thickness measurements to be made. Graft thickness was measured

with a micrometer at three points, one measurement was made from skin taken from each end of the skin graft strip. The third measurement was taken from skin that was between the two sections of the skin graft strip used to cover both defects. The split skin grafts were carefully applied with minimal tension and held in place with skin staples. The grafts were dressed according to the regimen allocated by opening an unmarked envelope which had the options of occlusive or conventional dressings inside. The occlusive dressing consisted of covering the split skin graft with Opsite and then covering this dressing with one layer of gauze dressings and securing these with a crepe bandage. The conventional dressing consisted of one layer of gauze dressings held with a crepe bandage. These dressings remained intact for 24 hours. They were then carefully removed (leaving the Opsite intact) and a check was made to identify haematoma or seroma under the grafts. If either of these two complications was detected in either the Opsite or the Conventional group the patient was removed from the study. Patients were reviewed approximately 7-10 days later and all dressings were removed. Further reviews were carried out at 1 month, 4-6 months and 12 months. During each occasion acetate tracings were made of the skin grafts.

Surface Area Estimation

The wound templates were transferred to an acetate sheet which was labelled. The surface area of the acetate tracings of all grafts was measured using a computerised digitising tablet. The edges were traced with a computerised mouse. This digitised the irregular shape and allowed the computer to calculate the surface area. The

result was expressed in square millimeters.

Stastical Analysis

The results were calculated as percentages of the initial wounding areas. The results were expressed as mean values with 90% confidence limits. The surface areas of the skin grafts in the Opsite groups were then compared to the areas in the control groups using the paired Student's t test.

5.2 Results

Failures

Twenty two patients with bilateral skin grafts entered the trial. When the grafts were inspected at 24 hours there was no sign of haematoma or seroma under any of the skin grafts. However, when the patients were seen at 7-10 post operative days six patients were excluded from the trial. The reasons for this were:

	Opsite Group	Conventional Group
Haematoma	1	2
Infection	1	1
Disturbed Graft		1

Twenty patients were reviewed at 1 month. This number fell to 12 patients presenting for review at 6 months and 11 patients at 12 months.

Skin Graft Thickness

The three measurements per sheet of split skin graft in the 16 patients entering the trial were tabulated. The mean graft thickness was 0.158 mm (90% confidence limits 0.149mm-0.168mm). These skin grafts would be classified as thin split skin grafts (Grabb and Smith 1979) and as such the wounds that they covered would be expected to contract

(Padgett 1942, Cronin 1961, Grabb and Smith 1979).

Surface Areas

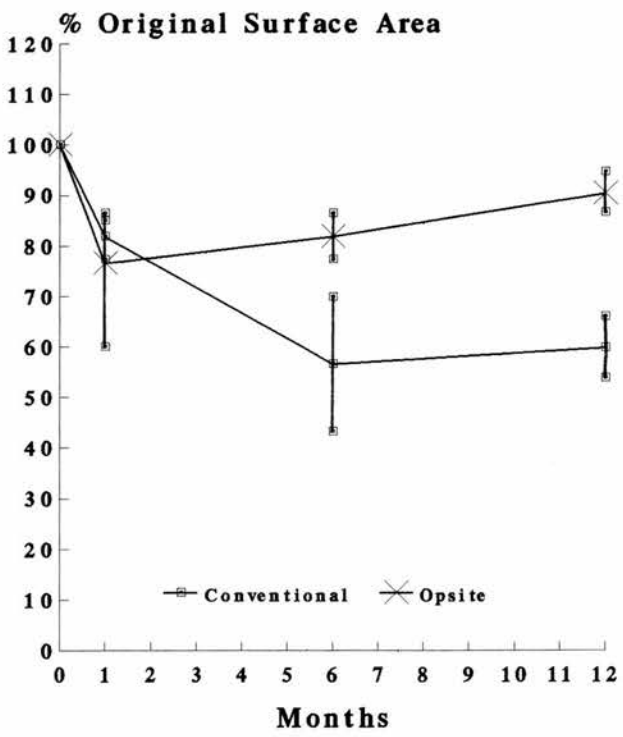
The graph below illustrates the results.

The skin grafted wounds from both the conventional and the Opsite covered group contracted to 81.8% and 76.5% their respective original surface areas in the first 4 weeks. There was no difference in the rate of contraction at this time ($p = 0.316$). At 6 months the conventionally dressed split skin grafts were 56.5% their original surface area whereas the Opsite covered skin grafts were 82% their original surface area. The mean difference between both groups was statistically significant ($p = 0.007$).

The surface areas at 12 months were 59.8% original surface area (conventional dressing) and 90.3% original surface area (Opsite dressing).

This difference was statistically significant ($p < 0.001$).

Human Skin Graft Wound Contraction



Part Four - Discussion

6.1 Discussion of Results

The studies in this thesis were designed to test the hypothesis that wound contraction is reduced when wounds are allowed to heal in a moist environment. Several parameters of wound healing were investigated to see if they were affected when the wound was covered with a moisture retaining (occlusive) synthetic dressing.

Human Study-Open Wounds

To date there has been little work carried out investigating the effects of a synthetic dressing on human wound contraction. The variety of full thickness wounds created make comparisons difficult and the ethical objections to leaving these wounds to heal by secondary intention are two main reasons for the paucity of such studies. Hien et al (1988) used the wounds resulting from Mohs micrographic surgery to study the effects of occlusive dressings on wound healing. These wounds were of mixed depth, size and shape and this made statistical analysis difficult. Hein's results indicated that the synthetic dressing was beneficial to epithelialisation and collagen metabolism. This study however relied on a subjective evaluation of the final scar and this evaluation compared wounds of differing depths that took different times to heal.

A study was therefore set up which would rely on an objective evaluation of wound contraction (size) in similar wounds from the same anatomical area. The wounds used in this study were split skin graft donor sites and

all wounds healed within 19 days, so standardising one part of the study. Although these wounds all include a portion of the dermis and epithelialisation is the major mode of healing, Alvarez et al (1983) has shown that collagen metabolism in superficial wounds is affected by wound dressings.

If collagen metabolism is affected do superficial wounds contract?

The findings of this study suggest that they do and if healing takes place in a moist environment this contraction is reduced. The study was designed to be a double blind investigation with the dressings being allocated after wounding and then removed (by another person) before the second measurement was made. The aim was to eliminate the need for biopsies to estimate wound depth, this would reduce the assault on the patient and eliminate the time consuming histological assessment. From the fact that all wounds had healed by 19 days (with no statistical difference between the two groups) and dressings had been randomly allocated, it can be assumed that both groups contained wounds of similar depth. This point has been ratified by a professional statistician.

The perhaps surprisingly high figure of 20% contraction in the conventionally dressed wounds is interesting. All wounds in this study were surrounded by normal, lax skin. Thus any physical force opposing wound contraction would be minimal (Sawhney 1977).

If the dressings were reducing wound contraction by their splintage action only, then one would expect the conventional dressing group to contract less because these

dressings become hard, are adherent to (and therefore splint) the wound until complete epithelialisation takes place. The synthetic dressing had minimal splintage so some other factor must have been responsible for reducing the wound contraction.

A reduction in moisture loss, an alteration in the wound surface bacterial counts, an alteration in the surface temperature or pizoelectric potentials have all been postulated as reasons for the dressing's effect. It was decided to investigate the effect on moisture loss to see if this factor was influenced when split skin grafted wounds were dressed with synthetic dressings.

Skin Grafted Wounds

In skin grafted wounds there is no exudate. Therefore, if an occlusive dressing was to have an effect on underlying wound contraction it must do so mainly by influencing the wound via the skin graft. The most likely way of doing so would be to reduce the dessicating stimulus by reducing water loss from the skin graft so allowing the underlying wound to heal in a moist environment.

Animal Study

Frank (1984,1985) and Foresman (1986) showed a reduction in wound contraction when "Biobrane", a nylon\silicone bilayer coated with porcine dermal polypeptides (Frank 1984) is used to dress the wound. In this study the synthetic dressing used was Opsite (a polyurethane membrane) with a split skin graft replacing the porcine dermal polypeptides. Full thickness wounds were created on a flexor and a non-flexor aspect on both sides of a rat.

This allowed in-animal controls so that paired results could be subsequently analysed using the Student's t test. Different groups of rats were studied with the Opsite dressing covering the skin graft for 1, 2, 3, 4 and 5 weeks. This time scale was chosen as maximum wound contraction occurs in rat skin grafted wounds during this time (Corps 1969). Myofibroblast numbers also undergo their biggest change before 5 weeks (Rudolph 1979, McGrath 1982, Darby et al 1990).

Moisture Vapour Transmission

The moisture vapour transmission (M.V.T.) of the open wounds, skin grafted wounds, skin grafted wounds synthetically dressed, and normal shaved skin was measured in this study.

When the first 88 wounds (in groups A15, B15, C15, D15 and E15) were created the M.V.T. was measured at a mean of 61.9 grams of water evaporated per square metre per hour (g/m2/hr), standard deviation (S.D.) 5.04 , normal skin had a M.V.T. of 5 g/m2/hr +/- S.D. 2. These results are similar to that (58 g/m2/hr +/- S.D. 6 for open wounds and 8 g/m2/hr +/- S.D. 2 for normal skin) found when Foresman (1986) carried out comparable studies in the rat with similar equipment.

The open wounds were then covered with a split skin graft taken from the skin removed in the creation of the original wound. Skin graft thickness was measured and all the skin grafts were similar. (Refer to appendix 2 for details)

Control Grafts

The M.V.T. fell to a mean value of 30 g/m2/hr (95%

confidence limit 29-31 g/m2/hr) when the grafts were applied. This finding was similar in all groups tested. (Appendix 3) The Moisture Vapour Transmission (M.V.T.) of the control split skin grafts was therefore approximately 3 times that of normal skin indicating that these wounds were being subjected to a continuing higher dessicating stimulus. This may be one reason why wounds covered with split skin grafts contract more than similar wounds covered with full thickness skin grafts (Corps 1969).

Opsite Covered Grafts

When the skin grafts were covered with Opsite the M.V.T. fell to approximately 15 g/m2/hr, about half that of the control wounds. There was no significant difference between the M.V.T. from the grafts on the flank compared to the grafts on the hip.

Dressing Removal

When the opsite dressing was removed from the split skin grafts there was a massive surge in M.V.T. to over 100 g/m2/hr. This value (equivalent to the M.V.T. of a partial thickness wound (Jonkman 1989) and double the M.V.T. of a freshly made full thickness wound) confirmed that the split skin grafted wound had healed in a very moist environment. The value quickly fell and at 30 minutes following the removal of the dressing the M.V.T. was similar to the values of the control grafts at the time of application. The following day the M.V.T. was checked and found to be similar to that of the opposite, control skin graft.

M.V.T. readings were similar irrespective of how long the skin graft had been covered with Opsite. The readings were

similar in Groups B,C,D and E, indicating that Opsite kept the wound healing environment very moist for as long as it remained in situ. There was no fluid between the graft and the dressing but the graft surface appeared very moist. There was no macroscopic evidence of any parakeratosis associated with normal graft revascularisation (Rudolph 1973) which was present on the opposite (control) grafts. This appearance is similar to what Jonkman (1989) found when superficial wounds re-epithelialised in extremely moist conditions. This effect according to Jonkman lasted for a few days, during which time the M.V.T. rate was increased. One day after the dressings were removed there was no statistical difference in M.V.T. between the control grafts and those that had been dressed with Opsite indicating that parakeratosis possibly influences moisture loss less in skin grafts than superficial wounds. The M.V.T. similarity between the control and the Opsite groups persisted to the end of the study (15 weeks post wounding).

Surface Area Estimation

Control Wounds

The control wounds in all groups behaved like skin grafts studied by other authors (Ragnell 1951, Corps 1969, Rudolph 1976, Sawhney 1977, Rudolph 1979). In this study, during the first 3-4 weeks the split skin grafts decreased in size until they were approximately 50-60% their original surface area. The initial rapid decrease in size that occurred in the grafts was not related to weight loss. All animals lost weight in the first post operative

week. (See Results - Rat Weights) After two weeks all animals in the study gained weight, and as surface area is related to body weight in the rat, the animal's surface area in general increased in size from 2-3 weeks onwards. When the surface area results are presented with rat weight standardized at 400g the initial reduction in size still takes place. (See Appendix ?)

After 4 weeks the skin grafts then increased in size reaching a value of 80-90% their original surface area at 15 weeks. There was no statistical evidence to suggest a difference (Group A, $p = 0.1017$) in the surface areas of the control groups in the flank and hip flexor area. Therefore it can be concluded that this area cannot be used as a model to study wound contraction in a flexor crease. This will be discussed in the conclusions section.

<u>Opsite Covered Groups</u>

The grafts covered with Opsite all contracted in a similar manner to the control grafts in the first 2-3 weeks after wounding. This contraction was not related to animal weight loss. When rat weight was standardized the initial reduction in size remained. (See appendix 3)

After 3 weeks the difference in the mean surface area of the synthetically dressed groups and the control groups became statistically significant ($p < 0.05$). This difference between the means persisted (with the exception of group D) with time to the end of the study. The largest mean surface area at 15 weeks was measured in group A15 at 164% the original wound's surface area. In groups B15(Opsite on for 2 weeks), C15(Opsite on for 3 weeks), D15(Opsite on for 4 weeks) and E15(Opsite on for 5

weeks) the mean values were 128%, 132%, 105% and 121% respectively. This indicates that if a split skin graft is covered with Opsite for only one week the underlying wound contraction process is altered, resulting in an increase in surface area to approximately twice that of the control group. Leaving the Opsite on the split skin graft for a longer period of time does not result in a further increase in size.

Although Frank (1985) showed that a wound covered with Biobrane reacted in a similar manner to a wound covered with a full thickness skin graft by reducing in size to only 90% it's original size at 17 days, other workers have shown varying degrees of wound contraction associated with full thickness skin grafts. Ragnell (1953), Corps (1969) and Sawhney (1971) found that wounds covered with a full thickness skin graft contracted to 80%, 65-80% and 60% respectively within 3-4 weeks of application. Therefore although the full thickness skin graft is considered the best clinical method for reducing wound contraction, during the first 3-4 weeks following wounding in the rat there is a reduction of wound size of between 20% and 40% when full thickness skin grafts are used. After the initial contraction phase of wound healing the wounds covered with full thickness skin grafts increase in size at a greater rate than those covered with split skin grafts.

The most marked difference between the effect full thickness skin grafts and split skin grafts have on wound contraction takes place after 3-4 weeks. This indicates that wound manipulation may not be able to fully reverse

the forces influencing wound contraction during this time. When the split skin grafts were covered with Opsite for one week (Group A), wound contraction in the first week was similar to that of the control wounds. When the dressing was removed the wound had contracted to 94% its original size. In the following three weeks the skin grafted wounds that had been covered with Opsite contracted at a slower rate than the in animal control wounds. Three weeks after graft application, the wounds had reduced to 68% their original size. This figure is comparable to that of full thickness skin graft covered wounds in other studies (see above). Thereafter, the skin grafted wounds of the Opsite group increased in size so that at the end of the study (15 weeks) the wounds were 164% their original size.

If one assumes that the period of wound healing after 4 weeks in the rat is predominantly the phase of collagen remodeling (Corps 1969) then it would seem that Opsite covered split skin grafts, like full thickness skin grafts have their most marked effect on this phase of wound contraction.

Hip Flexor Area

In order for this area to qualify as a model for wound contraction split skin grafted wounds must contract more that those on a non-flexor area. Evaluation of the surface area results of the control skin grafts in all groups did not show any significant difference from the control flank grafts.

Further comparison of the difference between control\Opsite grafts in the flank area and the difference

between the control\Opsite grafts in the hip area showed there to be no statistically significant difference between the flank and the hip areas.
In other words the site chosen in the hip flexor area was not a suitable model of a flexor crease.

Myofibroblast Cell Population

Evidence is continuing to accumulate to suggest that these cells, if not responsible for wound contraction are strongly associated with it. A recent review (Gown 1990) indicates that these cells may be derived from either fibroblasts or macrophages in the locality. These cells exhibit the characteristics of myofibroblasts because a smooth muscle phenotype has somehow been induced in them by the wound environment. How this occurs remains unclear but it would seem that the cytoplasmic constituents of myofibroblasts are constantly in a state of change. For example, in actively contracting wounds, the intermediate filaments of these cells contain actin, when wound contraction stops the intermediate filaments loose their actin components (Darby et al 1990). A monoclonal antibody was used to stain these cells by binding to the intra-cellular actin moiety of the intermediate filaments responsible for contraction. This has been shown to be successful when used with light microscopy by several authors (Gabbiani 1971, McGrath 1982). This technique allows larger sections to be processed giving a more accurate representation of myofibroblast numbers. However, the anti-actin antibody also stains myoepithelial cells (Franke et al 1980), vascular smooth muscle cells

(Gabbiani 1971) and perivascular pericytes (Darby et al 1990). To try to minimize these cells being erroneously counted as myofibroblasts the sections were counter stained with Heamotoxylin and Eosin to assess tissue architecture. If a cell that stained brown was not at the skin graft/wound bed interface it was not counted. If a cell was stained brown and it was in close approximation to a blood vessel then it was assumed that it was either a vascular smooth muscle cell or a perivascular pericyte and was excluded from the count. This was similar to the technique used by McGrath (1982) when she quantified myofibroblasts in open wounds. Individual cells were also not counted as they tended to clump together making single cell identification impossible.

The results from this study indicate that myofibroblast clumps peak when split skin graft surface area is at its smallest both in the control and the Opsite covered wounds. Although there is a statistical difference between the dressed and the control groups (see individual graphs for details) this difference remains constant from about 5 weeks onwards. Between 5 and 15 weeks the surface area in the Opsite skin grafts changes from 100% to 160% its original surface area. The myofibroblast clumps during this time change from 5 clumps per high powered field per unit surface area to approximately 1 clump per high powered field per unit surface area. This indicates that the myofibroblast numbers fall during the time the overlying skin grafts increase in size.

The design of the study however only allows statistical comparison between the Opsite covered grafts and the

control grafts within individual groups so further studies with greater numbers would be required to determine if there was a statistical correlation between myofibroblast numbers and change in wound size.

Human Split Skin Grafts

The results of this study indicate that dressing a split skin graft with an occlusive dressing alters wound contraction in a manner comparable to the reduction of wound contraction in the rat. Wound contraction in the control and the Opsite covered skin grafted wounds is similar in both groups one month after skin grafting. However, at 6 months after grafting, there is a statistically significant difference of 25% ($P = 0.007$) between the groups and at 12 months this difference is 30% ($P < 0.001$). It is interesting to note that dressing a split skin grafted wound during the first 7-10 days of wound healing can have an effect which manifests several months later, in the collagen remodeling phase of wound healing. This is the phase when scars expand in both animals and humans. The rate of expansion in this period appears to be related to body growth (Peacock and Van Winkle 1970) therefore one can expect the least expansion to occur in adults, the participants in this experiment.

The overriding aim of treatment of large burns is to reduce mortality. Surgical intervention is planned to convert the open burned area into a closed wound covered with viable epithelium. In burn wounds where little or no spontaneous reepithelialisation is anticipated, split skin autografts are routinely used to cover the defect. The major unfortunate consequence of this technique is the subsequent wound contraction which produces considerable cosmetic and functional disability especially in children. Attempts to reduce this contraction by late wound manipulation are labour intensive, costly and only partially successful.

Wound Contraction

Although wound contraction has been extensively studied in open wounds it probably occurs to a varying degree in all wounds. The human superficial wounds studied in this thesis did in fact contract within 2-3 weeks. Wound contraction is probably influenced by the amount of tissue lost during wounding. Even when no tissue is lost, as in incisional wounds, wound contraction still occurs in the scar subsequently formed.

When all of the dermis and epidermis is replaced (a full thickness skin graft) wound contraction is present in a reduced form. As one replaces less and less of the dermis (ie using thinner and thinner split skin grafts) wound contraction increases.

Therefore it would seem that the initial wounding events trigger the wound contraction process and subsequent treatment with current therapies can only reduce and not

eliminate this phenomenon.

Wound contraction is a dynamic phenomenon. It usually occurs when collagen is being laid down. In rats maximum contraction of skin grafted wounds occurs within 3-4 weeks following wounding. Thereafter the wounds increase in size, the ones covered with full thickness skin grafts enlarging more than those covered with split skin grafts. This event coincides with the time when incisional scars widen.

Wound Dressings

When an occlusive dressing was used to cover a superficial wound in humans it was found that it reduced wound contraction by a statistically significant amount in the first 2-3 weeks. This indicated the effect these dressings had on wound contraction could be repeated in humans.

The original papers by Frank indicated that a synthetic dressing could reduce wound contraction to the same extent as full thickness skin grafts.

When the split skin grafts in this thesis were covered with an occlusive dressing for only one week several findings were noted. The first was that the environment under the dressing was very humid, and remained so for as long as the dressing remained in situ. The surface area of the split skin grafts only began to differ statistically from the control grafts 3 weeks following wounding ie 2 weeks following dressing removal. The Opsite skin grafts then began to increase in size and at 15 weeks following wounding (14 weeks following dressing removal) the Opsite grafts were almost twice the size of the control skin grafts.

Split skin grafts covered with the occlusive dressing for longer than one week did not exhibit either a more marked reduction in initial contraction or a further subsequent increase in size indicating no benefit could be gained from the prolonged coverage.

The area chosen to represent a flexor crease did not differ from the non-flexor (flank) area with regards to the contraction tendencies of the skin grafts. There was no statistical difference between the sizes of the skin grafts at either site. The reason for this site failing to react like a flexor crease is probably because the wound was made on the flank aspect of the rat's thigh. Positioning the wound at the junctional area between the side of the animal and the ventral aspect of the leg may have resulted in greater success but this would have required a more obtrusive harness being in situ longer to prevent autocanabilisation. A dressing designed to protect a skin graft on the ventral aspect of the rat would not fully protect the skin graft on the flank area and may in fact interfere with this graft. The decision was therefore made to accept the lateral "flexor" wound site in the hope that it would function as a flexor area. This would mean no additional restrictions would be placed on the animal. The results indicate that this area cannot be used as a model of a flexor crease as wound contraction in this area does not differ from that in the flank area.

Human Study

The study was then repeated in human split skin grafted wounds with the graft covered with Opsite for only one week. The results indicated that there was no difference

between the grafts in the early phase of wound healing. However, at 6 and 12 months after wounding there was a statistically significant difference between both groups - the Opsite covered skin grafts being the larger.

Moist Wound Healing Effects

How do these dressings work?

When a wound is created the tissue environment at the surface of the wound has changed from being one which was bathed in interstitial fluid to one subjected to the dessicating stimulus of the outside atmosphere. The reduction of this stimulus may influence wound contraction by altering the processes of wound healing.

Little work has been done investigating the effects these dressings have on wound contraction. The majority of work has been carried out using open wounds as wound models. In these experiments, wound fluid accumulates under the dressing lifting it away from the wound surface. When dermis is present in the wound the initial wound contraction is reduced (eg. the superficial wounds studied in this thesis). When no dermis is present, however, wound contraction does not seem to be retarded when these dressings are used.

It could be argued from the above findings that the combination of moist wound healing with a portion of intact dermis results in a reduction of wound contraction.

One occlusive dressing (Biobrane) adheres to the wound surface via a membrane polypeptide-fibrin clot interaction, and this dressing does reduce the contraction

of full thickness wounds. In order for this dressing to work wound adhesion is mandatory and this adhesion has been likened to that of a skin graft. It would seem then that this dressing is the (temporary) synthetic equivalent of a full thickness skin graft with respect to reducing wound contraction. It is possible that the polypeptide layer on the undersurface of the dressing mimics dermal collagen whilst the outer nylon\silicone layers induce the moist environment.

A split skin autograft is a permanent source of dermal collagen.
When a wound is covered with a split skin graft which is then dressed with an occlusive dressing the wound healing environment is altered. During the period the graft is covered with the dressing the humidity at the skin graft surface is greatly increased. Water loss from the graft surface is therefore reduced so diminishing the dessicating stimulus to the underlying wound. Following the removal of the dressing the moisture vapour transmission rates return to those of the control grafts within 24 hours. This indicates that if these dressings are to have any effect on wound healing as a result of their influence on water loss they must do so only during the time they cover the graft. In this study the shortest period of time the skin grafts were covered with Opsite was one week.
The skin grafted wounds that have been covered with Opsite were always larger than their control equivalents. When Biobrane was removed from open wounds, contraction

continued albeit in a reduced form. The removal of Opsite from the skin grafts resulted in a similar reduction of wound contraction.

Skin grafted wounds that had been covered with Opsite virtually never contracted to the same extent as their controls. At the point of maximal contraction (3-4 weeks in rats) there was on average a 26% difference in surface area between the Grafts which had been covered with Opsite and the controls. This figure is similar to that found when other authors have compared the sizes of wounds covered with full thickness and split skin grafts.

The most marked difference between the Opsite Groups and the Control Groups occurs during the later stages of wound healing. At the end of the study (15 weeks post wounding) there was a mean difference of 42% between those grafts that had been covered with Opsite and those that had not. It is during this period of wound healing that the differences between full thickness and split skin grafts are most obvious.

From the data above it can be assumed that the early manipulation of a split skin grafted wound results in a reduction of wound contraction which manifests several weeks following dressing removal.

The time span of a healing wound in a human is greater than that of a rat. In burns patients many months may elapse before a split skin grafted wound stops contracting and enters the maturation phase of healing. It is therefore not surprising to note that it took longer for the differences between the skin grafted wounds covered with Opsite to manifest. The mean difference between the

Opsite covered grafts and the control grafts was 18% at 6 months and 23% at 12 months following surgery. The skin grafts covered with Opsite were still only 89% their original size at 1 year indicating that moist wound healing does not totally eradicate the stimulus or the effects of wound contraction.

Covering a split skin graft with an occlusive dressing during the early stages of wound healing has a significant effect on wound contraction. This effect persists when the dressing is removed and manifests maximally several months after its removal in rats as well as humans. It would seem likely therefore that the early manipulation of the wound environment may result in an alteration of the cells associated with wound contraction.

During the two decades since their identification data has continued to accumulate to suggest that myofibroblasts are the cells responsible for wound contraction. Recent studies suggest that smooth muscle actin manifests mainly in myofibroblasts present in actively contracting wounds. Therefore, the identification of these cells with an anti-actin antibody should give an accurate picture of the contracting capability of a wound.

The data collected for this work indicates that the numbers of myofibroblasts under a split skin graft are reduced when that graft is covered with an occlusive dressing. These numbers remain lower than the control wounds following dressing removal to the end of the study.

If the surface area data is combined with the myofibroblast data it would seem that:

A) myofibroblast numbers rise when wounds decrease in size and fall when wounds increase in size

B) The skin grafted wounds that contract less and increase in size to a greater degree contain a smaller numbers of myofibroblasts.

Crude cell counts may not be the complete answer to the quantification of the contractile force of the myofibroblasts. Their identification with an anti-actin antibody, although more accurate than pure morphological identification, also does not give the full answer. The immunocytochemical method does not differentiate between cells with varying quantities of actin in their cytoplasm. Myofibroblasts with different quantities of intracellular actin, and therefore (presumably) different contractile properties could not be differentiated with this method. However, to date, no other method of identification exists which is more accurate than the one used in this thesis.

An observed effect of Opsite dressings on open wound healing is the reduction of the inflammatory phase of wound healing. The human split skin grafts that had been covered with Opsite appeared to be paler and more pliable than the controls. The patients also commented that these grafts were less painful and itched less than the control grafts. These subjective findings do not contradict histological findings of a reduction of inflammation in wounds dressed with Opsite. One potential link between the reduction of inflammation and the reduction of wound

contraction via a reduction of myofibroblast numbers is the cytokine TGF beta. This mediator has been found to increase the inflammatory response, induce fibroplasia and alter cultured fibroblast phenotype to that of a wound healing fibroblast. It is possible that moist wound healing results in an alteration of the cytokine profile of a healing wound with perhaps a reduction in mediators such as TGF beta or other similar polypeptides.
This results in a diminished inflammatory response and a reduction of fibroplasia. When a wound is covered with a skin graft this leads to reduction in wound contraction.

Wound contraction is only reduced and not eliminated when early wound manipulation by occlusive dressings takes place. This is not surprising as wound contraction takes place when incisional wounds heal and when wounds are covered with full thickness skin grafts.
Incisional wounds by definition have no tissue loss so all events following wounding occur as a result of the initial wounding trauma and the responses to it. It would be of interest to investigate the m.v.t. rates of these wounds to see if there is a difference between water loss from traumatised skin and normal skin which may account for the subsequent contraction of incisional wounds.
The contraction that occurs in incisional wounds could therefore be regarded as "baseline wound contraction" and any attempt to reduce this contraction would require more active wound manipulation which may result in a disruption of the other aspects of normal wound healing. A reduction of this early wound contraction would be beneficial as

burn contractures can develop in the first few weeks following injury. This therefore occurs before the effect of moist wound healing manifests.

Summary

Wound contraction is a dynamic phenomenon which occurs in all wounds and can be reduced when the early wound healing environment is altered by the judicious use of occlusive dressings. Moist wound healing may be the initiating factor which results in a reduction of the inflammatory phase of wound healing one manifestation of which is a reduction in wound contraction.

It would seem that the presence of dermis is required for these dressings to work. Dermis is present in superficial wounds and wounds covered with a skin graft. When these wounds are covered with an occlusive dressing wound contraction is reduced.

These dressings only reduce and do not eliminate wound contraction. Their main effects manifest during the collagen remodelling phase of wound healing. This phase can occur late in human wound healing when contractures secondary to wound contraction may already exist.

The quantification of the cytokine profile of healing wounds of various types may provide baseline information apon which the effects of moist wound healing could be compared.

Wounds of differing types covered with different skin grafts could therefore be compared to see if there is a difference in cytokine concentrations accounting for the differences in wound contraction. If such differences

exist then specific cytokines could be neutralised by the addition of antibodies perhaps in the dressing so overcoming the fundemental tendency of the wound to contract as it heals.

Appendices

Appendix 1

Environmental Control

These are the environmental controls set in the rooms used during the experiments.

Temperature.....17-23 Centigrade
Relative Humidity.....40-60%
Lighting.....12 hours light, 12 hours dark cycle

Appendix 2 Split Skin Graft Thickness

	Rat No	Flank	hip
A15	1249	0.320	0.244
	1250	0.275	0.161
	1251	0.180	0.307
	1252	0.200	0.268
	1253	0.264	0.160
	1317	0.145	0.256
	1255	0.158	0.203
	1256	0.136	0.155
B15	1257	0.284	0.192
	1259	0.272	0.145
	1260	0.334	0.275
	1261	0.320	0.196
	1263	0.272	0.268
	1264	0.168	0.306
	1265	0.196	0.186
	1266	0.280	0.165
C15	1267	0.135	0.198
	1268	0.160	0.306
	1269	0.132	0.268
	1270	0.274	0.245
	1271	0.266	0.201
	1272	0.376	0.196
	1273	0.320	0.301
	1274	0.268	0.279
D15	1275	0.167	0.257
	1276	0.192	0.302
	1278	0.244	0.261
	1279	0.145	0.196
	1281	0.155	0.257
	1282	0.129	0.307
	1284	0.270	0.285
E15	1285	0.264	0.261
	1286	0.322	0.290
	1287	0.313	0.272
	1288	0.317	0.263
	1289	0.292	0.161
	1290	0.262	0.251
	1291	0.388	0.147
	1292	0.372	0.210
Mean		0.245	0.236
95% Confidence Limits		0.222 0.269	0.220 0.252

Paired t test

Degrees of Freedom	38	Mean Difference	0.009
t =	-0.577	95% Confidence	-0.040
p =	0.567	Limits	0.020

Appendix 3 - Animal Data

Rat Weights

Group A1

Rat Weights (in grams)

Rat No.	Opn.	Weeks after operation 1
1565	399	380
1566	465	409
1567	434	420
1568	477	456
1570	452	417
1573	480	458
1574	456	410
1575	486	457
Mn.Wt.	456	426
95% Confidence limits	475 438	444 407

Group A2

Rat Weights (in grams)

Rat No.	Opn.	Weeks after Operation 1	2
1372	430	382	410
1373	438	404	415
1376	435	400	407
1381	431	336	391
1382	463	423	436
1392	432	326	346
1397	461	425	430
1388	388	326	346
Mn.Wt.	435	378	398
95% confidence limits	450 420	405 350	420 375

Group A3

Rat Weights (in grams)

Rat No.	Opn	Weeks after operation 1	2	3
1552	434	361	407	420
1557	463	378	410	415
1559	408	339	388	401
1558	401	351	402	418
1561	464	371	434	440
1560	390	347	398	411
1562	371	297	356	379
1571	414	361	463	471
Mn.wt.	418	351	407	419
95% Confidence Limits	396 440	334 367	387 428	402 437

Group A4

Rat Weights (in grams)

Rat No.	Opn	Weeks after operation 1	2	3	4
1334	422	381	405	450	479
1335	369	348	356	382	402
1336	425	377	384	421	450
1337	373	384	394	407	459
1338	394	330	357	383	438
1339	421	335	360	408	407
1340	423	382	394	440	475
1341	479	324	371	397	432
Mn.wt.	413	358	378	411	443
95% Confidence Limits	390 436	341 374	365 390	395 427	424 461

Group A5

Rat Weights (in grams)

Rat No.	Opn.	Weeks after operation 1	2	3	4	5
1495	464	405	420	457	487	487
1498	428	388	397	438	466	483
1500	434	371	383	460	490	508
1513	438	368	381	463	506	525
1512	440	406	423	497	533	551
1510	421	380	399	410	514	535
1515	468	411	430	470	492	499
1517	390	352	368	391	415	423
Mn.wt.	435	385	400	448	488	501
95% Confidence Limits	419 451	371 399	386 415	426 470	465 511	476 527

Group A10

Rat Weights (in grams)

Rat No.	Opn.	Weeks after operation 1	2	3	4	5	6	7	8	9	10
1293	371	288	346	399	421	423	482	438	450	558	583
1294	403	358	401	438	463	480	509	504	522	539	560
1295	406	345	372	418	395	450	497	487	497	522	543
1296	416	363	393	447	469	488	503	508	515	536	562
1297	393	335	366	420	457	472	480	496	511	528	555
1298	407	364	389	434	457	477	478	498	511	531	549
1299	359	342	357	390	411	427	441	445	462	477	492
1300	367	316	335	374	402	422	437	455	463	480	501
Mn.Wt.	390	339	370	415	434	455	478	479	491	521	543
95% Confidence Limits	376 404	322 356	355 385	399 431	415 454	437 473	461 496	461 497	473 510	503 540	523 563

GROUP A15

Rat Weight (in grams)

Rat No.	Opn.	1	2	3	4	5	6	7	8	9
	Weeks After Operation									
1249	346	315	364	400	446	472	491	503	527	530
1250	352	312	344	377	417	443	466	487	506	510
1251	384	356	396	436	475	502	537	560	586	599
1252	358	307	363	389	421	436	453	471	472	479
1253	341	306	344	375	410	431	444	464	472	478
1317	379	357	384	387	392	414	435	467	470	492
1255	347	322	369	400	432	446	460	482	469	481
1256	323	294	340	381	420	443	465	484	500	506
Mean Wt.	354	321	363	393	427	448	469	490	500	509
95% Confidence Limits	341	306	350	380	410	431	448	470	474	483
	367	336	376	406	443	466	490	510	527	536

Rat No.	10	11	12	13	14	15
	Weeks After Operation					
1249	557	569	572	597	600	606
1250	530	543	547	562	572	582
1251	630	638	666	692	699	710
1252	495	504	501	510	510	522
1253	492	501	507	521	522	532
1317	509	521	540	555	564	575
1255	489	499	514	521	536	551
1256	520	531	541	562	562	578
Mean Wt.	528	538	549	565	571	582
95% Confidence Limits	497	508	514	527	532	544
	558	569	583	603	609	620

Group B2

Rat Weight (in grams)

Rat No.	Opn.	Weeks after Operation 1	2
1600	423	379	460
1601	455	416	400
1602	401	360	347
1603	455	407	410
1604	428	376	396
1605	450	405	406
1606	459	405	393
1511	412	373	403
Mn.Wt.	435	390	402
95% Confidence Limits	421	377	382
	450	403	422

Group B3

Rat Weight (in grams)

Rat No.	Opn	Weeks after operation 1	2	3
1572	455	402	400	410
1578	396	373	370	381
1579	361	344	392	400
1580	459	378	410	430
1581	393	331	355	567
1582	435	398	408	417
1583	464	410	421	439
1584	432	362	392	403
Mn.Wt.	424	375	394	431
95% Confidence Limits	400	356	379	393
	448	393	408	468

Group B4

Rat Weights (in grams)

Rat No.	Weeks after operation				
	Opn	1	2	3	4
1343	431	354	347	400	435
1344	424	363	354	400	428
1345	415	395	343	361	399
1346	422	409	390	407	424
1347	409	364	356	389	422
1348	394	370	357	392	421
1349	447	420	406	440	466
Mn.Wt.	420	382	365	398	428
95% Confidence Limits	409	365	348	382	414
	432	400	381	415	442

Group B5

Rat Weight (in grams)

Rat No.	Weeks after operation					
	Opn.	1	2	3	4	5
1524	424	374	376	419	445	459
1608	457	390	401	445	490	514
1609	432	370	392	401	431	461
1610	440	358	373	429	471	492
1611	337	380	399	380	415	433
1613	437	341	363	406	451	472
1614	432	361	393	431	480	506
1615	466	382	418	465	504	514
Mn.Wt.	428	370	389	422	461	481
95% Confidence Limits	403	359	378	405	441	462
	454	380	401	439	481	501

Group B10
Rat Weight (in grams)

Rat No.	Opn.	1	2	3	4	5	6	7	8	9	10
		Weeks after operation									
1301	341	358	404	416	425	442	463	461	498	493	517
1302	329	352	395	411	422	438	451	476	489	510	526
1303	369	380	395	422	432	457	410	490	504	525	552
1304	341	360	400	410	423	441	460	473	485	504	531
1305	359	378	409	435	459	480	500	517	582	505	523
1306	304	323	365	407	428	455	478	480	494	509	540
1307	310	325	358	391	415	431	444	471	485	506	532
1308	368	390	415	434	460	484	501	522	539	560	579
Mn.Wt.	340	358	393	416	433	454	463	486	510	514	538
95% Confidence Limits	324	342	379	406	422	441	444	472	487	501	525
	356	374	406	425	444	466	483	501	532	527	550

GROUP B15
Rat Weight (in grams)

Rat No.	Opn.	1	2	3	4	5	6	7	8	9
		WEEKS AFTER OPERATION								
1257	357	279	333	382	426	454	482	503	527	543
1259	348	322	351	374	424	440	457	473	489	501
1260	342	324	374	400	431	456	482	502	506	517
1261	357	320	359	497	437	465	489	514	536	542
1263	358	336	371	395	436	461	480	500	501	519
1264	347	326	339	371	405	420	430	452	457	461
1265	368	340	376	410	448	476	495	513	522	539
1266	350	340	365	384	416	435	446	471	460	473
Mn.Wt.	353	323	359	402	428	451	470	491	500	512
95% Confidence Limits	348	311	348	375	419	439	455	476	481	492
	359	336	369	428	437	463	485	506	519	532

Rat No.	Weeks After Operation 10	11	12	13	14	15
1257	561	573	577	578	579	591
1259	512	522	532	542	595	555
1260	560	581	549	563	569	575
1261	567	583	576	591	593	603
1263	530	543	550	554	560	565
1264	476	490	483	489	505	516
1265	553	572	587	610	616	631
1266	488	499	490	513	520	533
Mn.Wt.	531	545	543	555	567	571
95% Confidence Limits	508	521	518	529	542	547
	554	570	568	581	592	595

Group C3

Rat Weight (in grams)

Rat No.	Weeks after operation			
	Opn	1	2	3
1585	439	384	396	411
1586	411	361	376	391
1587	413	360	363	381
1590	434	390	366	391
1591	416	346	351	383
1593	374	312	361	374
1596	477	378	408	419
1598	413	390	424	441
Mn.Wt.	422	365	381	399
95% Confidence Limits	403	348	364	384
	441	382	397	414

Group C4

Rat Weights (in grams)

Rat No.	Weeks after operation				
	Opn	1	2	3	4
1350	445	397	386	388	381
1351	464	424	426	441	453
1352	430	398	347	390	397
1353	377	357	382	359	374
1357	456	391	401	421	419
1358	406	363	352	396	417
1359	469	431	407	412	453
1360	449	420	376	374	408
Mn.Wt.	437	398	385	398	413
95% Confidence Limits	417	380	367	381	394
	457	415	402	415	432

Group C5

Rat Weight (in grams)

Rat No.	Weeks after operation Opn.	1	2	3	4	5
1617	357	330	347	351	383	411
1618	473	426	435	459	484	508
1619	371	303	319	326	394	415
1620	446	388	400	427	457	430
1621	462	379	393	414	456	488
1563	461	439	449	356	469	478
1564	442	407	428	473	449	469
1569	442	398	403	446	475	499
Mn.Wt.	442	391	404	414	455	470
95% Confidence Limits	332	291	301	307	342	352
	553	491	507	522	568	587

Group C10

Rat Weight (in grams)

Rat No.	Weeks after operation Opn.	1	2	3	4	5	6	7	8	9	10
1309	406	368	375	393	405	431	460	466	471	484	500
1310	403	381	384	396	411	442	483	499	514	533	563
1311	388	363	340	353	373	397	413	418	446	465	482
1312	394	361	353	367	397	429	457	475	495	525	551
1312	382	350	340	376	381	404	442	437	447	463	473
1315	390	357	348	360	372	395	419	425	446	470	493
1316	412	379	320	377	388	430	487	478	495	517	533
Mn.Wt.	398	368	353	378	393	421	451	458	475	495	515
95% Confidence Limits	391	360	338	367	382	408	431	437	456	474	491
	406	376	368	389	404	434	471	479	494	516	539

GROUP C15

Rat Weight (in grams)

Rat No.	Opn.	Weeks After Operation 1	2	3	4	5	6	7	8	9
1267	356	324	333	358	387	403	420	449	442	450
1268	359	320	314	347	375	397	423	453	454	467
1269	321	271	316	342	351	373	387	402	404	406
1270	366	343	296	287	327	362	388	313	273	340
1271	373	356	349	355	390	415	434	470	470	474
1272	381	326	369	378	407	435	464	492	500	512
1273	345	302	243	266	306	355	387	430	426	430
1274	363	334	364	371	405	434	458	490	492	509
Mn Wt	358	322	323	338	369	397	420	437	433	449
95% Confidence Limits	346	305	296	312	345	377	400	399	386	412
	370	339	350	364	392	417	440	475	479	485

Rat No.	Weeks After Operation 10	11	12	13	14	15
1267	466	475	485	508	510	513
1268	484	500	494	506	507	524
1269	406	446	418	428	430	449
1270	390	425	406	329	297	350
1271	494	515	521	535	537	582
1272	529	542	556	570	572	610
1273	449	476	466	484	484	517
1274	521	540	538	561	562	547
Mean Wt	467	490	486	490	487	512
95% Confidence Limits	435	462	451	439	430	459
	500	517	520	541	545	564

GROUP D4

Rat Weights (in grams)

Rat No.	Weeks after Operation				
	Opn	1	2	3	4
1361	406	408	381	366	363
1362	471	461	425	429	436
1363	450	410	380	401	412
1364	397	363	362	370	365
1365	454	422	412	414	441
1366	451	430	428	428	450
1367	437	393	416	439	438
1368	460	391	390	420	423
Mn.Wt.	441	410	399	408	416
95% Confidence Limits	424	391	384	391	394
	458	429	415	426	438

Group D5

Rat Weight (in grams)

Rat No.	Weeks after Operation					
	Opn.	1	2	3	4	5
1532	394	339	339	418	420	418
1533	335	295	306	327	351	396
1534	381	328	364	378	396	422
1535	393	350	352	368	384	392
1536	385		Died			
1537	360	362	350	360	387	415
1539	389	362	385	340	350	421
1540	355	321	350	342	370	398
Mn.Wt.	374	337	349	362	380	409
95% Confidence Limits	360	320	333	341	363	400
	388	353	366	383	397	418

Group D10

Rat Weight (in grams)

Rat No.	Opn.	Weeks after wounding 1	2	3	4	5	6	7	8	9	10
1318	378	319	355	370	357	389	423	442	465	481	479
1319	435	380	394	415	420	431	438	503	530	547	561
1320	380	337	396	419	417	422	456	469	495	523	514
1321	376	322	360	384	387	400	439	460	481	500	504
1322	353	320	344	357	360	387	400	417	437	451	453
1323	403	346	375	403	412	425	471	491	512	523	542
1324	430	322	371	410	424	437	472	494	507	533	539
1325	396	336	374	372	375	390	439	455	475	487	485
Mn.Wt.	398	368	353	378	393	421	451	458	475	495	515
95% Confidence Limits	380 417	355 381	341 365	363 393	375 411	408 434	435 467	439 477	456 494	474 516	491 539

GROUP D15

Rat Weight (in grams)

Rat No.	Opn.	Weeks After Operation 1	2	3	4	5	6	7	8	9
1275	365	344	346	372	380	381	419	459	479	500
1276	390	347	360	384	433	434	460	490	502	520
1278	345	334	328	359	372	391	462	455	471	483
1279	427	376	282	330	375	420	486	499	523	543
1281	406	376	368	385	411	444	456	525	504	568
1282	382	357	366	393	423	417	492	488	520	534
1284	392	371	395	421	434	432	453	482	565	584
Mn.Wt	387	358	349	378	404	417	461	485	509	533
95% Confidence Limits	368 405	346 369	325 374	358 397	385 423	401 433	445 478	469 502	488 531	509 558

Rat No.	Weeks After Operation 10	11	12	13	14	15
1275	523	547	550	568	570	590
1276	542	573	570	574	576	584
1278	512	539	539	555	559	571
1279	579	597	610	632	634	641
1281	596	607	629	630	648	678
1282	560	579	592	606	610	619
1284	612	623	630	667	681	696
Mn.Wt	561	581	589	605	611	626
95% Confidence Limits	535 586	560 602	563 614	577 633	580 642	592 659

Group E5

Rat Weight (in grams)

Rat No.	Weeks after wounding Opn	1	2	3	4	5
1541	431	385	376	413	441	445
1544	369	339	339	350	342	403
1548	360	336	322	341	356	363
1549	361	318	331	349	349	387
1550	398	342	355	386	437	452
1556	402	372	373	411	446	455
Mn.Wt.	387	349	349	375	395	418
95% Confidence Limits	366	330	333	351	358	389
	408	367	366	399	432	446

Group E10

Rat Weight (in grams)

Rat No.	Weeks after wounding Opn.	1	2	3	4	5	6	7	8	9	10
326	426	379	394	405	422		422	463	496	511	516
327	378	356	363	407	351		396	433	460	477	491
328	389	351	381	381	385		411	438	460	479	487
329	458	396	412	427	440	not	465	493	519	538	557
330	391	355	381	417	423	done	465	484	514	534	542
332	445	399	388	412	396		437	470	497	511	528
333	438	384	361	393	392		433	446	473	487	502
Mn.Wt.	418	374	383	406	401		433	461	488	505	518
95% Confidence Limits	396	360	371	396	381		415	445	472	488	500
	439	388	395	416	422		451	477	505	523	536

Group E15

Rat Weight (in grams)

Rat No.	Weeks After Operation									
	Opn.	1	2	3	4	5	6	7	8	9
1324	389	325	367	406	415	409	441	503	529	558
1287	397	346	368	366	397	408	437	459	487	510
1288	368	327	361	375	376	378	400	430	477	473
1289	368	333	357	342	326	347	372	428	450	476
1290	373	313	297	358	372	374	400	448	470	495
1291	356	311	350	383	387	390	415	421	438	455
1292	344	303	329	335	318	320	360	391	410	431
1317	409	342	403	422	450	451	471	487	500	518
Av.Wt.	376	325	354	373	380	385	412	446	470	490
95% Confidence Limits	362	315	334	354	352	359	388	422	446	464
	389	335	374	393	408	411	436	470	494	515

Rat No.	Weeks After Operation					
	10	11	12	13	14	15
1324	572	608	633	654	671	692
1287	530	550	566	570	582	601
1288	491	488	506	512	519	537
1289	492	530	544	562	576	590
1290	510	518	536	546	560	579
1291	469	462	446	458	492	515
1292	460	458	466	485	496	509
1317	531	538	543	547	554	570
Mn.Wt.	507	519	530	542	556	574
95% Confidence Limits	483	487	492	503	519	536
	531	551	568	580	594	612

PILOT STUDY

This study compared the results of 2 methods of measuring the surface area of a 2x2cm acetate block.

The columns labelled shape contain the results of photographing the shape through a video camera and projecting that image on a computer screen. This image was traced with an electronic mouse and the surface area of the traced shape calculated by the computer.

The columns labelled tracing contain data resulting from the initial tracing of the 2x2cm acetate block onto an acetate sheet.The surface area of the traced shape was then calculated using the above method.

The units used are mm^2.

Tracing	Shape	Tracing	Shape
396	400	384	395
400	415	356	391
378	392	358	371
321	389	381	408
350	416	387	406
368	387	369	412
357	394	375	400
325	378	374	387
349	409	388	381
410	410	397	392
369	420	392	379
378	387	384	388
375	375	364	396
381	377	366	407
395	398	356	393
378	406	368	422
365	408	372	431
369	403	384	378
369	379	395	385
370	386	367	399
372	381	352	386
365	389	361	405
352	392	365	406
367	394	391	385
369	399	400	402
378	420		

	Tracing	Shape
mean	372.39	396.25
95% confidence Limits	367.64 377.15	392.53 399.98
%error	6.90	0.94

Pilot Study - Moisture Vapour Transmission

		M.V.T. RATES (g/m2/hr)			
	Rat No	FLANK skin	FLANK wound	HIP skin	HIP wound
A15	1249	5.30	59.30	5.20	69.00
	1250	6.20	69.30	4.60	58.90
	1251	8.90	70.90	8.20	78.30
	1252	5.10	78.60	9.10	69.20
	1253	10.20	68.50	7.60	82.10
	1317	9.60	69.10	7.50	83.60
	1255	8.40	70.10	6.20	56.10
	1256	5.30	56.90	3.90	69.80
B15	1257	5.90	69.00	7.10	74.40
	1259	5.60	69.90	9.20	69.00
	1260	8.10	59.30	4.80	75.60
	1261	7.30	74.30	5.90	78.40
	1263	7.20	68.90	6.10	80.30
	1264	6.80	68.10	6.90	59.00
	1265	5.50	85.30	7.10	60.90
	1266	4.90	79.50	7.00	63.20
C15	1267	8.90	81.10	5.00	94.00
	1268	7.30	56.30	6.30	89.20
	1269	9.50	69.20	6.90	75.60
	1270	9.60	67.50	4.90	69.00
	1271	8.10	64.90	7.00	80.10
	1272	5.60	73.10	8.10	78.20
	1273	7.60	82.10	7.60	65.90
	1274	5.90	78.50	5.90	65.80
D15	1275	6.30	69.40	6.80	63.00
	1276	9.10	73.20	6.00	89.40
	1278	9.90	65.30	7.00	76.00
	1279	10.50	89.00	7.50	68.00
	1281	11.20	62.90	7.90	59.90
	1282	4.90	68.30	8.40	74.40
	1284	8.30	74.20	8.10	69.60
E15	1285	7.80	79.60	6.00	96.20
	1286	11.10	65.30	8.50	58.30
	1287	4.90	56.30	7.30	67.90
	1288	6.20	89.00	6.90	80.00
	1289	9.50	78.60	9.00	78.30
	1290	7.30	81.50	9.80	65.00
	1291	7.90	58.10	5.80	71.00
	1292	8.40	65.90	3.90	80.10
	mean	7.59	70.93	6.85	72.89
	95% Confidence Limits	7.01	68.24	6.40	69.81
		8.17	73.62	7.29	75.97

Moisture Vapour Transmission Results

The following tables are derived from measurements of moisture vapour transmission measured with an evaporimeter.

M.V.T. is measured in grams of water per meter squared per hour (g\m2\Hr).

The time (in weeks) the measurements are made is indicated in the row at the top of the table.

The letters after the numbers refer to the times immediately following dressing removal

a.....1 minute following Opsite removal
b.....10 minutes following Opsite removal
c.....20 minutes following Opsite removal

There is a column labelled 1d in Group A15 comprising of data recorded 24 hours following Opsite removal. The rats in this group took a long time to recover from the anaesthetic and a proportion required (temporary) ventilatory support. As a result of this no further anaesthetics were given within 24 hours of a lengthy anaesthetic.

The tables comprise of data from the flank wounds and the hip wounds.

At the bottom of each section are 3 rows.
The mean values and 95% confidence limits for the mean values are contained therein.

GROUP A 15 Flank

Rat No.	Time (Weeks)							
	0	1	1a	1b	1c	1d	2	3
Control Wounds								
1249	30.5	29.0	27.7	23.0	26.5	30.0	25.0	22.0
1250	31.9	29.7	25.7	23.6	25.2	29.9	24.7	19.9
1251	23.0	32.3	25.5	24.2	29.9	22.1	25.9	21.3
1252	34.2	24.1	26.6	25.6	25.0	23.0	27.1	24.7
1253	28.6	25.7	26.1	20.7	25.0	26.7	27.8	18.4
1255	34.7	25.8	23.1	22.9	24.9	31.0	25.8	21.2
1256	21.5	28.6	22.0	21.0	21.8	23.6	24.8	23.0
mean	29.5	28.2	25.7	23.0	24.8	26.1	25.4	21.3
95% cl	26.3 – 32.7	26.4 – 29.9	24.2 – 27.2	22.0 – 24.0	22.8 – 26.7	23.6 – 28.5	24.3 – 26.5	20.0 – 22.6

	Weeks			
	4	5	10	15
1249	17.0	15.0	14.0	13.0
1250	16.4	15.9	13.3	11.0
1251	19.9	12.9	19.0	17.1
1252	15.5	17.9	15.3	16.3
1253	15.7	16.1	14.7	12.9
1255	15.0	14.5	13.1	11.4
1256	16.0	17.1	14.4	13.7
mean	16.3	15.5	14.6	13.4
95% cl	15.2 – 17.3	14.5 – 16.5	13.3 – 15.9	12.0 – 14.9

	Time (Weeks)							
	0	1	1a	1b	1c	1d	2	3
Opsite Wounds								
1249	16.0	15.8	105.6	87.7	33.9	29.8	22.5	20.1
1250	15.9	14.9	110.4	89.5	37.7	25.1	23.1	20.0
1251	16.7	15.5	98.9	76.9	29.4	27.9	24.9	24.7
1252	18.0	17.4	100.0	90.8	30.6	31.1	21.0	25.1
1253	14.7	12.9	104.5	85.3	35.3	33.9	19.3	17.9
1255	15.5	15.3	103.8	91.1	40.0	27.0	24.0	20.2
1256	16.7	15.1	100.0	76.9	32.9	23.1	25.8	22.1
mean	16.2	15.5	103.3	84.9	34.5	28.3	23.3	21.0
95% cl	15.6 – 16.8	14.6 – 16.4	100.9 – 105.8	81.0 – 88.7	32.2 – 36.8	26.1 – 30.5	21.8 – 24.8	19.3 – 22.8

	Weeks			
	4	5	10	15
1249	18.3	15.6	13.7	12.2
1250	21.8	15.0	12.9	12.0
1251	17.9	18.0	12.1	11.9
1252	12.3	14.9	16.4	16.8
1253	18.1	13.3	15.8	12.5
1255	14.3	16.1	15.9	11.1
1256	23.6	19.9	12.3	10.0
mean	17.7	16.1	14.4	12.5
95% cl	15.2 – 20.1	14.7 – 17.4	13.2 – 15.5	11.2 – 13.8

Group A15 Hip

Rat No.	Time (Weeks) 0	1	1a	1b	1c	1d	2	3
Control Wounds								
1249	34.7	23.5	24.3	24.9	26.9	26.4	20.4	18.6
1250	32.2	24.8	23.6	25.0	27.1	26.3	23.2	19.9
1251	33.2	34.9	25.4	25.4	26.6	26.8	21.5	20.0
1252	35.4	32.1	26.1	26.8	26.2	25.1	24.9	17.3
1253	26.9	33.2	26.3	23.5	25.9	23.4	23.7	18.0
1255	32.9	29.8	23.4	24.3	21.9	21.4	22.2	17.9
1256	28.7	26.9	26.8	23.3	26.0	25.5	21.8	17.7
mean	31.5	29.3	25.4	25.2	25.6	25.5	22.7	18.5
95% cl	29.4 – 33.6	26.6 – 31.9	24.4 – 26.3	24.1 – 26.2	24.5 – 26.7	24.0 – 27.0	21.7 – 23.6	17.8 – 19.1

	Weeks 4	5	10	15
1249	16.2	14.3	14.0	13.5
1250	15.0	15.0	12.0	12.3
1251	17.8	15.5	13.6	15.6
1252	18.3	13.3	13.5	14.9
1253	16.6	14.4	13.9	15.8
1255	14.5	16.9	15.0	12.9
1256	16.3	15.3	11.1	13.6
mean	16.2	14.9	13.5	14.0
95% cl	15.4 – 17.1	14.2 – 15.6	12.6 – 14.3	13.2 – 14.8

Rat No.	Time (Weeks) 0	1	1a	1b	1c	1d	2	3
Opsite Wounds								
1249	15.3	16.1	100.3	78.9	55.6	45.9	26.0	19.3
1250	16.1	15.4	105.3	85.2	61.2	32.2	23.5	18.6
1251	14.3	15.7	104.9	83.3	67.7	36.1	21.1	18.0
1252	14.9	14.7	105.5	84.9	63.0	38.0	22.2	19.1
1253	15.5	16.6	107.6	79.2	59.4	30.0	24.9	16.6
1255	16.5	16.5	110.2	81.3	69.1	39.0	25.0	17.3
1256	18.2	14.6	100.0	84.3	63.7	33.7	27.3	18.0
mean	15.8	15.6	104.1	81.0	62.0	36.4	24.2	17.9
95% cl	15.0 – 16.5	15.0 – 16.1	101.4 – 106.7	78.0 – 84.1	58.9 – 65.2	33.3 – 39.6	22.9 – 25.5	17.1 – 18.6

	Weeks 4	5	10	15
1249	13.4	15.0	11.2	12.5
1250	16.0	16.5	13.6	12.6
1251	15.9	18.0	14.9	12.7
1252	17.8	13.6	14.4	11.3
1253	17.0	14.0	13.2	13.9
1255	15.5	16.2	16.0	12.0
1256	16.1	18.6	12.7	15.9
mean	15.8	16.0	13.7	13.1
95% cl	14.9 – 16.7	14.8 – 17.1	12.7 – 14.6	12.2 – 14.1

Group B15

Rat No.	Time (Weeks) 1	2	2a	2b	2c	3	4	5	10	15
Flank										
Control Wounds										
1257	30.3	25.3	24.0	25.3	24.4	20.1	19.3	15.3	12.6	10.0
1259	29.6	26.1	23.6	26.5	24.1	22.6	14.9	12.6	11.5	13.6
1260	28.6	28.3	25.9	23.9	23.7	23.3	17.6	16.4	13.6	11.8
1261	31.6	24.3	24.0	24.8	23.5	24.0	17.3	16.3	12.0	12.0
1263	31.7	25.5	24.3	26.0	24.9	25.9	17.8	17.0	12.9	12.3
1264	33.4	26.1	25.1	25.1	26.1	26.7	19.2	14.9	11.8	10.9
1265	28.9	26.4	29.0	23.0	28.3	23.5	18.6	15.5	12.6	10.8
mean	30.1	25.9	24.9	24.8	25.0	23.2	17.6	15.4	12.6	11.3
95% cl	28.8 31.5	25.1 26.6	23.7 26.1	24.0 25.6	24.0 26.0	21.6 24.8	16.6 18.6	14.5 16.3	12.1 13.1	10.4 12.2
Opsite Wounds										
1257	16.2	15.8	100.7	78.6	59.0	25.6	19.3	16.3	13.3	9.3
1259	15.6	12.9	105.3	79.2	63.4	28.3	15.9	15.2	12.6	11.2
1260	13.6	13.3	104.0	69.1	65.1	23.1	17.7	19.0	15.6	9.6
1261	17.6	14.7	106.3	70.8	62.0	25.6	18.3	15.8	14.0	12.5
1263	16.0	14.4	104.5	84.1	58.6	25.0	18.5	15.3	13.9	13.0
1264	15.9	16.5	100.9	80.6	59.1	24.9	18.6	16.7	13.7	13.8
1265	15.5	15.5	101.5	81.1	66.0	28.9	17.3	16.0	14.1	12.7
mean	15.6	14.8	103.4	77.8	62.2	26.4	18.1	15.9	13.8	11.8
95% cl	14.9 16.4	14.0 15.6	102.0 104.7	74.5 81.2	60.3 64.1	24.9 28.0	17.4 18.8	14.8 17.0	13.2 14.4	10.8 12.9
Hip										
Control Wounds										
1257	33.3	29.8	26.9	25.9	25.4	24.7	17.9	13.5	16.3	8.3
1259	31.0	25.3	25.8	26.8	30.6	21.6	17.3	16.9	18.9	12.6
1260	29.6	24.6	27.9	28.3	24.6	20.0	18.6	15.7	12.3	11.2
1261	28.9	24.5	23.2	26.4	28.1	19.9	19.3	15.4	15.6	10.2
1263	31.2	23.1	26.8	27.3	27.6	19.1	19.1	14.3	17.6	13.5
1264	30.0	27.0	25.9	23.1	27.7	18.7	18.6	19.3	18.0	13.0
1265	33.0	22.3	26.0	26.8	26.9	22.6	21.1	12.0	16.3	12.9
mean	30.7	25.0	26.2	26.7	27.4	21.6	18.7	15.0	16.0	11.8
95% cl	29.6 31.8	23.5 26.6	25.3 27.1	25.6 27.8	26.2 28.5	19.8 23.3	17.9 19.5	13.5 16.5	14.4 17.5	10.6 13.0
Opsite Wounds										
1257	16.3	15.9	106.1	64.3	45.0	25.6	25.6	14.5	12.0	12.0
1259	15.1	15.7	105.4	66.0	36.2	23.4	13.4	16.5	13.1	13.4
1260	18.3	19.4	110.0	65.3	39.1	21.0	19.8	15.9	15.9	12.8
1261	16.4	13.6	105.6	68.1	37.9	29.4	17.0	16.4	13.8	10.6
1263	16.9	15.4	104.6	69.1	35.2	21.3	16.9	17.0	12.9	11.8
1264	15.4	13.8	107.7	75.3	43.1	24.8	17.9	16.8	13.7	8.4
1265	18.7	16.5	106.3	59.1	41.2	24.0	18.4	15.9	14.5	12.5
mean	16.8	15.7	105.8	66.7	40.3	24.0	18.5	16.0	13.9	11.3
95% cl	15.9 17.6	14.5 16.8	104.1 107.5	63.7 69.6	37.8 42.7	22.2 25.7	16.3 20.7	15.4 16.6	13.1 14.7	10.2 12.5

Group C15

Time (Weeks)

Rat No.	1	2	3	3a	3b	3c	4	5	10	15
Flank										
Control Wounds										
1268	31.2	26.3	19.5	15.8	15.5	15.9	13.5	15.3	11.9	7.8
1269	32.9	28.7	20.3	15.3	15.0	15.3	14.9	10.5	12.0	12.5
1270	29.6	29.0	26.0	15.2	14.9	15.0	12.0	11.6	13.1	10.9
1271	26.5	25.3	21.0	16.9	15.9	16.2	13.4	13.9	12.8	11.1
1272	28.9	23.6	21.7	13.4	13.4	13.8	16.8	12.5	11.7	10.2
1274	29.6	23.4	21.3	12.0	12.5	13.0	12.9	11.0	9.5	9.2
mean	30.0	25.7	21.6	15.5	15.3	15.5	13.9	12.7	11.7	10.1
95% cl	28.7 31.2	24.2 27.1	20.1 23.0	14.1 16.8	14.1 16.6	14.4 16.6	13.0 14.8	11.6 13.8	11.0 12.5	9.1 11.0
Opsite Wounds										
1268	15.9	14.9	14.2	110.9	75.6	56.8	26.4	13.7	12.8	9.8
1269	15.3	14.7	14.8	102.5	79.2	68.1	21.5	19.8	12.9	7.3
1270	14.6	13.5	15.2	103.5	73.1	43.5	25.7	18.7	13.4	12.5
1271	14.9	15.8	15.6	106.4	69.5	59.1	23.9	17.9	17.0	12.4
1272	15.9	17.3	14.3	109.9	78.1	55.5	20.1	17.8	12.5	12.9
1274	18.6	16.3	12.3	107.3	69.4	57.6	20.7	15.9	16.0	8.4
mean	16.0	15.8	14.3	108.0	74.9	56.8	22.9	17.0	13.9	11.0
95% cl	15.2 16.8	14.7 16.9	13.7 15.0	105.6 110.3	72.1 77.7	52.4 61.2	21.4 24.4	15.8 18.3	12.9 15.0	9.5 12.4
Hip										
Control Wounds										
1268	31.2	26.4	20.8	19.5	21.0	20.4	18.2	13.4	16.1	11.8
1269	33.5	24.3	23.0	22.5	22.6	22.0	21.6	14.9	13.5	12.9
1270	29.2	25.6	19.5	20.6	20.1	21.3	15.2	17.2	13.8	13.4
1271	28.7	29.4	17.9	18.9	16.8	17.3	18.0	15.6	11.4	13.6
1272	26.5	21.0	20.0	19.6	19.4	18.9	16.7	15.4	12.7	14.1
1274	32.1	25.2	23.9	22.5	23.4	21.9	19.5	14.1	12.8	10.7
mean	29.5	25.2	20.8	20.9	20.9	20.5	18.5	15.3	13.3	12.5
95% cl	27.4 31.5	23.6 26.7	19.5 22.1	20.0 21.9	19.4 22.3	19.1 21.8	17.2 19.8	14.5 16.0	12.4 14.1	11.7 13.3
Opsite Wounds										
1268	16.2	16.1	14.6	98.7	79.6	49.8	19.4	15.9	16.5	11.2
1269	14.9	15.3	12.0	110.3	82.4	42.1	18.7	15.0	15.0	11.5
1270	14.5	14.7	13.9	109.6	80.6	39.5	16.6	16.0	15.8	9.5
1271	14.3	13.9	13.7	108.6	79.6	38.7	19.6	14.3	15.7	10.7
1272	15.4	14.1	14.2	102.6	75.6	45.7	20.0	14.9	13.9	10.1
1274	13.6	13.6	14.5	100.7	89.2	41.6	21.0	15.8	16.7	11.9
mean	15.0	15.0	14.0	104.8	79.5	43.6	20.3	15.6	15.2	10.9
95% cl	14.4 15.5	14.3 15.7	13.4 14.6	101.8 107.7	76.2 82.7	41.2 46.1	18.8 21.8	15.1 16.0	14.5 15.9	10.3 11.6

Group D15

Time (Weeks)

Rat No.	1	2	3	4	4a	4b	4c	5	10	15
					Flank					
	Control Wounds									
1275	33.2	25.3	23.5	18.9	18.6	18.7	19.0	17.0	14.9	10.6
1276	32.1	23.5	24.1	18.7	18.5	17.9	18.3	17.9	13.7	11.3
1278	30.6	23.7	24.5	20.6	17.9	19.3	18.5	20.6	15.0	11.4
1279	34.3	25.1	25.9	22.1	21.9	21.6	22.0	21.0	15.6	11.9
1281	32.9	25.6	26.1	19.3	20.9	19.9	21.0	20.6	15.3	12.5
1282	30.0	24.9	22.6	18.3	19.8	19.5	19.9	19.2	13.9	13.1
1284	31.2	24.7	22.8	19.7	19.3	18.5	19.3	17.9	12.9	13.6
mean	32.0	24.7	24.2	19.7	19.6	19.3	19.7	19.2	14.5	12.1
95%	31.0	24.1	23.3	18.8	18.6	18.5	18.8	18.1	13.8	11.3
cl	33.1	25.2	25.2	20.6	20.5	20.2	20.6	20.3	15.1	12.8
	Opsite Wounds									
1275	15.3	14.6	16.5	13.5	109.3	80.6	39.6	22.1	16.8	11.0
1276	15.6	15.3	13.6	14.6	106.9	81.3	34.9	22.5	18.3	11.9
1278	16.3	13.7	15.9	15.9	104.9	73.0	43.9	23.5	16.4	12.6
1279	16.9	15.8	15.1	15.2	105.2	76.9	41.2	19.6	19.3	14.3
1281	14.9	15.8	15.3	13.5	103.3	75.1	43.6	18.3	13.7	15.0
1282	15.9	16.3	14.6	14.9	107.8	79.3	44.4	22.3	14.6	15.9
1284	16.0	16.5	15.0	14.1	107.3	85.6	37.1	23.8	15.5	16.3
mean	15.8	15.4	15.1	14.5	106.4	78.8	40.7	21.7	16.4	13.9
95%	15.4	14.7	14.5	13.9	105.0	75.9	38.2	20.3	15.0	12.5
cl	16.3	16.1	15.8	15.1	107.8	81.7	43.2	23.1	17.7	15.3
					Hip					
	Control Wounds									
1275	31.2	28.6	22.6	21.3	21.6	20.8	21.5	18.6	16.8	12.0
1276	33.1	27.3	23.5	20.0	20.9	19.3	21.0	14.9	15.9	12.9
1278	29.6	23.5	24.9	25.6	24.3	25.0	24.1	17.4	15.3	13.8
1279	28.7	25.9	21.8	25.5	23.9	24.5	24.3	19.0	14.6	14.1
1281	25.6	25.4	24.3	24.9	22.0	20.6	23.6	18.0	13.9	14.5
1282	28.6	28.7	25.9	28.7	26.2	25.9	27.6	19.5	18.7	13.2
1284	30.1	25.6	21.8	20.0	19.6	20.3	19.1	19.5	17.7	13.9
mean	29.6	26.4	23.5	23.7	22.6	22.3	23.0	18.1	16.1	13.5
95%	28.0	25.1	22.5	21.4	21.1	20.5	21.1	17.0	15.0	12.9
cl	31.2	27.7	24.6	26.0	24.2	24.2	24.9	19.2	17.3	14.1
	Opsite Wounds									
1275	14.3	15.3	13.6	13.5	104.9	80.6	35.6	26.8	16.9	13.6
1276	15.6	14.9	13.7	13.1	106.7	79.1	39.4	23.4	19.4	10.3
1278	15.2	14.7	15.9	14.8	105.3	83.2	38.9	25.9	19.8	11.9
1279	15.9	16.3	15.2	14.3	103.8	76.1	45.8	27.3	18.3	13.5
1281	15.0	12.9	13.8	14.5	106.8	75.9	41.6	27.1	18.7	13.9
1282	19.1	17.3	14.6	13.7	105.9	77.8	32.9	25.8	16.4	13.9
1284	14.7	16.2	14.3	13.6	110.0	73.9	33.7	26.2	17.9	9.1
mean	15.7	15.4	14.4	13.9	106.2	78.1	38.3	26.1	18.2	12.3
95%	14.6	14.4	13.9	13.5	104.8	75.9	35.1	25.2	17.3	11.0
cl	16.8	16.3	15.0	14.3	107.6	80.3	41.4	27.0	19.1	13.6

Group E15

Time (Weeks)

Rat No.	1	2	3	4	5	5a	5b	5c	10	15
					Flank					
	Control Wounds									
1317	30.2	25.6	23.5	18.6	15.6	13.5	13.9	13.3	11.2	10.1
1324	33.6	26.5	22.6	19.6	16.0	14.0	13.8	14.2	12.6	9.6
1287	31.6	23.9	25.9	17.3	16.9	14.9	15.8	14.5	10.8	9.3
1288	29.3	24.8	23.5	18.6	14.3	13.5	14.3	13.3	11.4	10.5
1289	26.9	27.9	24.1	18.4	15.6	14.5	14.0	13.9	11.1	11.8
1290	28.7	27.1	24.6	16.9	17.1	13.6	13.9	13.7	12.6	10.3
1291	31.8	26.7	24.9	18.3	13.6	12.9	13.5	13.1	11.9	9.6
1292	30.7	26.1	23.4	17.0	14.5	12.1	13.0	12.5	10.3	8.0
mean	30.4	26.1	24.1	18.1	15.5	13.6	14.0	13.6	11.5	9.9
95%	29.0	25.2	23.4	17.5	14.6	13.1	13.5	13.1	11.0	9.2
cl	31.7	26.9	24.7	18.7	16.3	14.2	14.6	14.0	12.0	10.6
	Opsite Wounds									
1317	15.3	14.9	13.9	15.6	13.6	106.3	69.5	29.3	12.9	13.5
1324	16.4	13.8	14.5	14.3	14.3	109.2	68.7	26.8	11.0	12.4
1287	14.7	15.6	14.6	13.6	15.1	103.4	73.6	25.9	13.4	13.9
1288	15.3	16.2	15.0	13.2	14.9	105.7	74.5	26.7	12.9	11.0
1289	16.9	16.8	13.6	12.9	13.6	103.5	72.1	29.8	12.7	10.6
1290	18.3	16.7	13.8	12.7	12.9	100.5	70.0	30.4	13.8	10.9
1291	15.4	12.3	12.7	13.5	13.1	101.9	79.6	28.1	10.5	9.5
1292	15.7	13.6	13.4	11.0	13.0	109.4	78.1	29.9	11.1	10.0
mean	16.0	15.0	13.9	13.4	13.8	105.0	73.3	28.4	12.3	11.5
95%	15.2	13.9	13.5	12.5	13.3	102.9	70.7	27.2	11.5	10.4
cl	16.8	16.0	14.4	14.2	14.4	107.1	75.9	29.5	13.1	12.5
					Hip					
	Control Wounds									
1317	33.2	26.5	26.9	19.6	18.6	18.6	17.9	17.5	13.9	11.6
1324	36.0	23.8	20.1	18.3	18.3	18.9	18.0	18.6	13.7	12.8
1287	32.5	28.4	21.3	18.2	17.3	18.3	18.6	19.0	14.6	13.4
1288	31.9	26.1	20.5	18.0	16.9	18.5	17.3	16.7	18.4	13.5
1289	28.7	25.0	20.6	17.6	17.4	16.5	17.9	15.8	12.6	12.7
1290	29.6	23.1	25.1	19.1	16.8	16.5	17.0	16.1	13.7	9.5
1291	29.8	22.1	20.3	21.0	13.6	14.5	15.0	13.9	12.8	10.3
1292	30.0	24.7	21.8	20.0	16.7	16.5	15.7	16.3	11.6	12.7
mean	31.5	25.0	22.1	19.0	17.0	17.3	17.2	16.7	13.9	12.1
95%	29.9	23.7	20.4	18.2	16.0	16.3	16.4	15.7	12.6	11.1
cl	33.0	26.3	23.7	19.7	17.9	18.3	18.0	17.8	15.2	13.0
	Opsite Wounds									
1317	14.9	15.3	14.7	14.9	15.2	101.5	89.3	33.6	15.6	16.8
1324	16.3	16.7	15.6	15.0	13.6	104.9	78.1	34.9	12.8	12.4
1287	16.8	13.8	14.5	13.5	14.3	109.7	75.0	26.8	13.4	9.8
1288	15.2	16.7	14.5	13.9	13.1	106.4	74.9	25.9	11.2	13.4
1289	15.9	15.4	15.1	13.9	12.8	105.5	73.5	36.1	12.9	15.7
1290	13.4	13.4	14.9	14.2	12.1	106.7	69.5	32.5	12.4	14.6
1291	14.9	15.0	13.0	13.0	15.1	103.8	78.1	33.0	13.0	13.8
1292	16.7	14.6	15.1	14.6	13.9	110.2	83.4	27.4	10.8	11.0
mean	15.5	15.1	14.7	14.1	13.8	106.1	77.7	31.3	12.8	13.4
95%	14.8	14.3	14.2	13.7	13.1	104.2	73.7	28.7	11.8	11.9
cl	16.3	15.9	15.2	14.6	14.5	108.0	81.7	33.8	13.7	15.0

Statistical Analysis of M.V.T. Results

The following tables compare the Opsite covered skin grafts with the controls using paired Student's "t" tests.

Group A15

Flank

	Time (weeks) 0	1	1a	1b	1c	1d
Degrees of Freedom	6	6	6	6	6	6
"t"	-6.401	-10.307	52.013	28.609	4.507	0.821
"p"	0.0007	0.000	0.000	0.000	0.004	0.443
Mean Difference	-12.99	-12.62	78.01	62.46	8.79	1.66
95%	-17.95	-15.61	74.40	57.12	4.02	-3.28
cl	- 8.02	-9.62	81.74	67.80	13.56	6.60

	Time (weeks) 2	3	4	5	10	15
Degrees of Freedom	6	6	6	6	6	6
"t"	-2.382	-0.089	1.041	0.443	-0.565	-1.467
"p"	0.055	0.932	0.339	0.680	0.593	0.193
Mean Difference	-2.93	-0.06	1.54	0.49	-0.67	-1.27
95%	-5.94	-1.63	-2.09	-2.26	-3.58	-3.39
cl	0.08	1.52	5.17	3.23	2.24	0.85

Hip

	Time (weeks) 0	1	1a	1b	1c	1d
Degrees of Freedom	6	6	6	6	6	6
"t"	-10.951	-8.430	48.571	62.745	16.517	5.611
"p"	0.000	0.000	0.000	0.000	0.000	0.001
Mean Difference	-16.17	-13.66	79.70	57.70	37.01	11.43
95%	-19.78	-17.66	75.69	55.45	31.53	6.44
cl	-12.56	-9.65	83.71	59.95	42.50	16.41

	Time (weeks) 2	3	4	5	10	15
Degrees of Freedom	6	6	6	6	6	6
"t"	1.511	-0.700	-0.782	1.834	0.677	-1.464
"p"	0.182	0.510	0.464	0.116	0.524	0.193
Mean Difference	1.76	-0.36	0.43	1.03	0.41	-1.10
95%	-1.09	1.61	-1.77	-0.34	-1.08	-2.94
cl	4.60	0.89	0.91	2.40	1.91	0.74

Group B15

Flank

	Time (weeks) 1	2	2a	2b	2c	3
Degrees of Freedom	6	6	6	6	6	6
"t"	-27.868	-14.186	59.007	26.377	30.874	1.755
"p"	0.000	0.000	0.000	0.000	0.000	0.130
Mean Difference	-14.81	-11.27	78.19	52.70	36.89	2.19
95%	-16.12	-13.22	74.95	47.81	33.96	-0.86
cl	-13.51	-9.33	81.42	57.59	39.81	5.23

	Time (weeks) 4	5	10	15
Degrees of Freedom	6	6	6	6
"t"	0.396	1.482	7.249	0.133
"p"	0.760	0.189	0.004	0.899
Mean Difference	0.13	0.90	1.46	0.10
95%	-0.67	-0.59	0.97	-1.74
cl	0.92	2.39	1.95	1.94

Hip

	Time (weeks) 1	2	2a	2b	2c	3
Degrees of Freedom	6	6	6	6	6	6
"t"	-19.641	-7.314	124.022	17.423	6.676	2.642
"p"	0.000	0.000	0.000	0.000	0.000	0.038
Mean Difference	14.27	-9.47	80.46	40.37	12.40	3.27
95%	-16.05	-12.64	78.87	34.70	7.86	-0.24
cl	-12.49	-6.30	82.04	46.04	16.94	6.30

	Time (weeks) 4	5	10	15
Degrees of Freedom	6	6	6	6
"t"	-0.279	1.072	-2.282	-0.029
"p"	0.790	0.325	0.063	0.978
Mean Difference	-0.41	0.84	-2.73	-0.03
95%	-4.05	1.08	-5.65	-2.45
cl	3.22	2.77	0.20	2.40

Group C15

Flank

	Time (weeks) 1	2	3	3a	3b	3c
Degrees of Freedom	5	5	5	5	5	5
"t"	-13.610	-7.055	-7.774	55.060	33.921	13.083
"p"	0.000	0.001	0.001	0.000	0.000	0.000
Mean Difference	-13.92	-10.63	-7.23	91.98	59.62	41.90
95%	-16.55	-14.51	-9.63	87.69	55.10	33.67
cl	-11.29	-6.76	-4.84	96.28	64.13	50.13

	Time (weeks) 4	5	10	15
Degrees of Freedom	5	5	5	5
"t"	5.624	3.225	2.215	0.223
"p"	0.003	0.023	0.078	0.832
Mean Difference	9.13	4.83	2.27	0.27
95%	4.96	0.98	0.36	-2.80
cl	13.31	8.69	4.92	3.34

Hip

	Time (weeks) 1	2	3	3a	3b	3c
Degrees of Freedom	5	5	5	5	5	5
"t"	-13.324	-9.111	-6.643	40.868	44.413	12.420
"p"	0.000	0.000	0.001	0.000	0.000	0.000
Mean Difference	-15.38	-10.70	-7.03	84.48	60.62	22.60
95%	-18.35	-13.72	-9.76	79.17	57.11	17.92
cl	-12.42	-7.68	-4.31	89.80	64.13	27.28

	Time (weeks) 4	5	10	15
Degrees of Freedom	5	5	5	5
"t"	1.206	0.339	3.495	-2.313
"p"	0.281	0.748	0.017	0.069
Mean Difference	1.02	0.22	2.22	-1.93
95%	-1.15	-1.43	0.59	-4.08
cl	3.18	1.86	3.85	0.21

Group.D15

Flank

	Time (weeks) 1	2	3	4	4a	4b
Degrees of Freedom	6	6	6	6	6	6
"t"	-25.706	-25.358	-15.033	-11.688	76.171	31.626
"p"	0.000	0.000	0.000	0.000	0.000	0.000
Mean Difference	16.20	-9.26	-9.07	-5.13	86.73	59.49
95% cl	-17.74	-10.15	-10.55	-6.20	83.94	54.88
	-14.76	-8.36	-7.59	-4.05	89.51	64.09

	Time (weeks) 4c	5	10	15
Degrees of Freedom	6	6	6	6
"t"	16.573	2.114	2.465	4.604
"p"	0.000	0.079	0.049	0.004
Mean Difference	20.96	2.56	1.90	1.80
95% cl	17.86	-0.40	0.01	0.84
	24.05	5.52	3.79	2.76

Hip

	Time (weeks) 1	2	3	4	4a	4b
Degrees of Freedom	6	6	6	6	6	6
"t"	-12.022	-16.440	-14.689	-8.604	57.277	41.527
"p"	0.000	0.000	0.000	0.000	0.000	0.000
Mean Difference	-13.87	-11.06	-9.10	-9.79	83.64	55.74
95% cl	-16.69	-12.70	-10.62	-12.57	80.07	52.46
	-11.05	-9.41	-7.58	-7.00	87.22	59.03

	Time (weeks) 4c	5	10	15
Degrees of Freedom	6	6	6	6
"t"	7.871	20.352	2.012	-1.446
"p"	0.000	0.000	0.090	0.198
Mean Difference	15.24	7.94	2.07	-1.17
95% cl	10.50	6.99	-0.45	-3.15
	19.98	8.90	4.59	0.81

Group E15

Flank

	Time (weeks)					
	1	2	3	4	5	5a
Degrees of Freedom	7	7	7	7	7	7
"t"	-14.586	-15.111	-20.764	-13.105	-3.376	75.783
"p"	0.000	0.000	0.000	0.000	0.012	0.000
Mean Difference	-14.35	-11.09	-10.13	-4.74	-1.69	91.38
95% cl	-16.68 -12.02	-12.82 -9.35	-11.28 -8.97	-5.59 -3.88	-2.78 -0.49	88.52 94.23

	Time (weeks)			
	5b	5c	10	15
Degrees of Freedom	7	7	7	7
"t"	39.238	19.759	1.499	2.278
"p"	0.000	0.000	0.177	0.057
Mean Difference	59.24	4.80	0.80	1.58
95% cl	55.67 62.81	13.03 16.57	-0.46 2.06	-0.06 3.21

Hip

	Time (weeks)						
	1	2	3	4	5	5a	5b
Degrees of Freedom	7	7	7	7	7	7	7
"t"	-19.282	-11.675	-8.190	-9.510	-4.418	78.296	26.520
"p"	0.000	0.000	0.000	0.000	0.003	0.000	0.000
Mean Difference	-15.59	-9.85	-7.40	-4.85	-3.19	88.96	60.55
95% cl	-17.91 -13.99	-11.84 -9.86	-9.54 -5.26	-6.06 -3.64	-4.89 -1.48	86.28 91.65	55.16 65.94

	Time (weeks)			
	5b	5c	10	15
Degrees of Freedom	7	7	7	7
"t"	26.520	8.920	-1.231	1.187
"p"	0.000	0.000	0.258	0.274
Mean Difference	60.55	14.54	-1.15	1.38
95% cl	55.16 65.94	10.68 18.39	-3.36 1.06	-1.37 4.12

Surface Areas

Group A2 Flank

OPSITE					CONTROL				
Rat No.	Wd. No.	Wnd	Bx.	% Diff	Rat No.	Wd. No.	Wnd	Bx	% Diff
1382	1	445	342	76.9	1382	3	461	375	81.3
1372	1	314	423	134.7	1372	3	461	162	35.1
1376	1	420	305	72.6	1376	3	438	355	81.1
1381	3	412	340	82.5	1381	1	408	243	59.6
1388	3	455	249	54.7	1388	1	395	112	28.4
1397	1	440	238	54.1	1397	3	451	296	65.6
1373	3	404	334	82.7	1373	1	401	223	55.6
Mean Difference				79.7	Mean Difference				58.1
Confidence Limits (95%)				61 98	Confidence Limits (95%)				44 72

Group A2 Hip

OPSITE					CONTROL				
Rat No.	Wd. No.	Wnd	Bx.	% Diff	Rat No.	Wd. No.	Wnd	Bx	% Diff
1382	2	420	317	75.5	1293	4	433	166	38.3
1372	4	486	334	68.7	1294	2	409	191	46.7
1376	2	487	370	76.0	1295	4	515	173	33.6
1381	4	457	167	36.5	1296	2	466	242	51.9
1388	2	515	307	59.6	1297	4	504	242	48.0
1397	4	520	239	46.0	1298	2	497	228	45.9
1373	2	497	337	67.8	1299	4	451	282	62.5
Mean Difference				61.4	Mean Difference				46.7
Confidence Limits (95%)				51 72	Confidence Limits (95%)				40 53

Group A3 Flank

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1341	3	427	390	91.3	1293	1	394	300	76.1
1340	1	416	269	64.7	1294	3	401	134	33.4
1339	3	488	354	72.5	1295	1	398	159	39.9
1338	1	411	310	75.4	1296	3	426	300	70.4
1337	3	450	132	29.3	1297	1	349	187	53.6
1336	1	415	295	71.1	1298	3	378	185	48.9
1335	3	312	180	57.7	1299	1	373	106	28.4
1334	3	432	357	82.6	1300	1	369	107	29.0
Mean Difference				68.1	Mean Difference				47.5
Confidence Limits (95%)				56 80	Confidence Limits (95%)				36 59

Group A3 Hip

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1293	2	413	232	56.2	1293	4	466	142	30.5
1294	4	425	144	33.9	1294	2	378	60	15.9
1295	2	434	233	53.7	1295	4	379	192	50.7
1296	4	385	273	70.9	1296	2	412	150	36.4
1297	2	438	149	34.0	1297	4	469	117	24.9
1298	4	380	223	58.7	1298	2	407	173	42.5
1299	2	366	246	67.2	1299	4	404	266	65.8
Mean Difference				53.5	Mean Difference				38.1
Confidence Limits (95%)				43 64	Confidence Limits (95%)				27 50

Group A4 Flank

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1552	1	398	309	77.6	1552	3	362	203	56.1
1557	3	383	328	85.6	1557	1	440	262	59.5
1559	1	462	299	64.7	1559	3	369	302	81.8
1558	3	384	376	97.9	1558	1	429	255	59.4
1561	1	507	398	78.5	1561	3	420	161	38.3
1560	3	416	562	135.1	1560	1	455	418	91.9
1562	1	369	346	93.8	1562	3	417	178	42.7
1571	3	414	423	102.2	1571	1	428	252	58.9
Mean Difference				91.9	Mean Difference				91.9
Confidence Limits (95%)				78.1 105.7	Confidence Limits (95%)				78.1 105.7

Group A4 Hip

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1552	2	437	362	82.8	1552	4	392	181	46.2
1557	4	442	260	58.8	1557	2	432	167	38.7
1559	2	486	347	71.4	1559	4	486	292	60.1
1558	4	437	316	72.3	1558	2	415	272	65.5
1561	2	499	377	75.6	1561	4	443	145	32.7
1560	4	468	433	92.5	1560	2	465	283	60.9
1562	2	486	253	52.1	1562	4	379	107	28.2
1571	4	360	378	105.0	1571	2	494	210	42.5
Mean Difference				76.3	Mean Difference				91.9
Confidence Limits (95%)				65.2 87.4	Confidence Limits (95%)				78.1 105.7

Group A5 Flank

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1495	1	383	265	69.2	1495	3	362	207	57.2
1500	1	392	410	104.6	1500	3	435	354	81.4
1510	3	362	432	119.3	1510	1	356	312	87.6
1512	1	370	360	97.3	1512	3	353	162	45.9
1511	3	546	505	92.5	1511	1	495	373	75.4
1515	1	370	401	108.4	1515	3	417	178	42.7
Mean Difference				96.6	Mean Difference				69.5
Confidence Limits (95%)				84.1 109.1	Confidence Limits (95%)				55.6 83.4

Group A5 Hip

Rat No.	OPSITE Wd. No.	Wound	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wound	Bx	% Diff
1495	2	505	374	74.1	1495	4	400	289	72.3
1500	2	425	399	93.9	1500	4	366	298	81.4
1510	4	397	397	100.0	1510	2	343	285	83.1
1512	2	427	302	70.7	1512	4	424	197	46.5
1511	4	464	494	106.5	1511	2	478	424	88.7
1515	2	487	449	92.2	1515	4	445	376	84.5
Mean Difference				89.0	Mean Difference				74.4
Confidence Limits (95%)				78.6 99.4	Confidence Limits (95%)				63.1 85.7

Group A10 Flank

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1293	1	365	409	112.1	1293	3	367	305	83.1
1294	3	378	484	128.0	1294	1	334	272	81.4
1295	1	368	576	156.5	1295	3	309	357	115.5
1296	3	329	325	98.8	1296	1	348	321	92.2
1297	1	346	650	187.9	1297	3	328	394	120.1
1298	3	346	529	152.9	1298	1	344	286	83.1
1299	1	348	477	137.1	1299	3	354	194	54.8
1300	3	412	393	95.4	1300	1	428	215	50.2
Mean Difference				133.6	Mean Difference				85.1
Confidence Limits (95%)				113 154	Confidence Limits (95%)				69 101

Group A10 Hip

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1293	2	467	471	100.9	1293	4	366	273	74.6
1294	4	365	332	91.0	1294	2	386	257	66.6
1295	2	364	516	141.8	1295	4	280	351	125.4
1296	4	311	517	166.2	1296	2	337	306	90.8
1297	2	415	402	96.9	1297	4	368	372	101.1
1298	4	362	436	120.4	1298	2	283	316	111.7
1299	2	414	537	129.7	1299	4	360	220	61.1
1300	4	402	356	88.6	1300	2	290	226	77.9
Mean Difference				116.9	Mean Difference				88.6
Confidence Limits (95%)				99 135	Confidence Limits (95%)				74 103

Group A15 Flank

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1249	1	413	564	136.6	1249	3	367	305	83.1
1250	3	230	262	113.9	1250	1	334	272	81.4
1251	1	282	613	217.4	1251	3	309	357	115.5
1252	3	283	484	171.0	1252	1	348	321	92.2
1253	1	237	382	161.2	1253	3	328	394	120.1
1255	1	190	433	227.9	1255	1	344	286	83.1
1256	3	229	273	119.2	1256	3	354	194	54.8
Mean Difference				163.9	Mean Difference				90.1
Confidence Limits (95%)				133 195	Confidence Limits (95%)				75 105

Group A15 Hip

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1249	2	320	391	122.2	1249	4	469	214	45.6
1251	2	377	596	158.1	1251	4	294	390	132.7
1252	4	345	473	137.1	1252	2	396	352	88.9
1253	2	296	320	108.1	1253	4	325	234	72.0
1255	2	352	280	79.5	1255	4	352	405	115.1
1256	4	264	285	108.0	1256	2	335	258	77.0
Mean Difference				118.8	Mean Difference				88.5
Confidence Limits (95%)				99 139	Confidence Limits (95%)				66 111

Group B2 Flank

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1600	3	366	224	61.2	1600	1	363	320	88.2
1602	1	406	431	106.2	1602	3	345	365	105.8
1603	1	415	374	90.1	1603	3	380	409	107.6
1606	1	390	302	77.4	1606	3	403	312	77.4
1605	3	442	344	77.8	1605	1	394	270	68.5
1604	1	406	267	65.8	1604	3	400	205	51.3
1511	1	414	399	96.4	1511	3	421	240	57.0
Mean Difference				82.1	Mean Difference				79.4
Confidence Limits (95%)				71 93	Confidence Limits (95%)				64 95

Group B2 Hip

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1600	4	383	308	80.4	1600	2	439	291	66.3
1601	4	409	379	92.7	1601	2	457	234	51.2
1602	2	428	315	73.6	1602	4	401	197	49.1
1603	2	447	324	72.5	1603	4	499	397	79.6
1606	2	402	362	90.0	1606	4	414	278	67.1
1605	4	420	250	59.5	1605	2	466	383	82.2
1604	2	469	411	87.6	1604	4	382	271	70.9
1511	2	350	365	104.3	1511	4	468	199	42.5
Mean Difference				82.6	Mean Difference				63.6
Confidence Limits (95%)				74 92	Confidence Limits (95%)				54 73

Group B3 Flank

	OPSITE				CONTROL			
Rat No.	Wd. No.	Wnd	Bx.	% Diff	Rat No.	Wd. No.	Wnd	Bx	% Diff
1512	1	438	311	71.0	1512	3	403	287	71.2
1578	3	361	394	109.1	1578	1	315	279	88.6
1581	1	449	342	76.2	1581	3	389	233	59.9
1582	3	430	404	94.0	1582	1	445	312	70.1
1583	1	417	413	99.0	1583	3	469	302	64.4
1584	3	406	321	79.1	1584	1	401	208	51.9
Mean Difference				88.1	Mean Difference				67.7
Confidence Limits (95%)				77 99	Confidence Limits (95%)				59 77

Group B3 Hip

	OPSITE				CONTROL			
Rat No.	Wd. No.	Wnd	Bx.	% Diff	Rat No.	Wd. No.	Wnd	Bx	% Diff
1512	2	473	366	77.4	1512	4	416	268	64.4
1578	4	341	341	100.0	1578	2	391	299	76.5
1581	2	482	356	73.9	1581	4	430	183	42.6
1582	4	378	314	83.1	1582	2	402	212	52.7
1583	2	412	407	98.8	1583	4	438	216	49.3
1584	4	371	357	96.2	1584	2	432	251	58.1
Mean Difference				88.2	Mean Difference				57.3
Confidence Limits (95%)				80 97	Confidence Limits (95%)				48 66

Group B4 Flank

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	CONTROL Rat No.	Wd. No.	Wnd	Bx	% Diff
1343	3	425	257	60.5	1343	1	397	200	50.4
1344	1	314	381	121.3	1344	3	352	248	70.5
1345	3	410	294	71.7	1345	1	426	146	34.3
1346	1	369	293	79.4	1346	3	358	126	35.2
1347	3	443	409	92.3	1347	1	416	358	86.1
1348	1	354	276	78.0	1348	3	378	211	55.8
1349	3	440	203	46.1	1349	1	376	175	46.5
Mean Difference				78.5	Mean Difference				54.1
Confidence Limits (95%)				62 95	Confidence Limits (95%)				41 67

Group B4 Hip

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	CONTROL Rat No.	Wd. No.	Wnd	Bx	% Diff
1343	4	397	313	78.8	1343	2	477	378	79.2
1344	2	407	387	95.1	1344	4	411	277	67.4
1345	4	406	234	57.6	1345	2	386	154	39.9
1346	2	371	262	70.6	1346	4	331	181	54.7
1347	4	432	439	101.6	1347	2	373	299	80.2
1348	2	462	402	87.0	1348	4	424	201	47.4
1349	4	428	208	48.6	1349	2	391	119	30.4
Mean Difference				77.1	Mean Difference				57.0
Confidence Limits (95%)				64 90	Confidence Limits (95%)				44 70

Group B5 Flank

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1524	1	436	397	91.1	1524	3	376	275	73.1
1610	3	370	277	74.9	1610	1	396	294	74.2
1611	1	409	284	69.4	1611	3	361	239	66.2
1614	3	419	405	96.7	1614	1	416	309	74.3
1608	1	393	540	137.4	1608	3	426	323	75.8
1613	3	383	368	96.1	1613	1	412	322	78.2
1615	1	398	369	92.7	1615	3	444	188	42.3
Mean Difference				94.0	Mean Difference				69.2
Confidence Limits (95%)				79 109	Confidence Limits (95%)				61 78

Group B5 Hip

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1524	2	363	428	117.9	1524	4	464	331	71.3
1610	4	317	334	105.4	1610	2	246	216	87.8
1611	2	438	382	87.2	1611	4	365	217	59.5
1614	4	392	486	124.0	1614	2	433	289	66.7
1608	2	370	412	111.4	1608	4	365	305	83.6
1613	4	436	467	107.1	1613	2	422	313	74.2
Mean Difference				108.8	Mean Difference				73.8
Confidence Limits (95%)				100 118	Confidence Limits (95%)				66 82

Group B10 Flank

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1302	3	307	377	122.8	1302	1	335	312	93.1
1303	1	375	387	103.2	1303	3	347	371	106.9
1304	1	284	304	107.0	1304	3	306	241	78.8
1305	1	317	319	100.6	1305	3	361	192	53.2
1306	3	350	558	159.4	1306	1	355	421	118.6
1307	1	370	424	114.6	1307	3	370	236	63.8
1308	3	355	399	112.4	1308	1	324	281	86.7
Mean Difference				117.2	Mean Difference				85.9
Confidence Limits (95%)				103 131	Confidence Limits (95%)				70 102

Group B10 Hip

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1302	4	376	377	100.3	1302	2	343	300	87.5
1303	2	333	393	118.0	1303	4	338	215	63.6
1304	2	318	318	100.0	1304	4	364	270	74.2
1305	2	263	327	124.3	1305	4	312	205	65.7
1306	4	310	539	173.9	1306	2	338	390	115.4
1307	2	380	366	96.3	1307	4	404	270	66.8
1308	4	346	402	116.2	1308	2	305	240	78.7
Mean Difference				118.4	Mean Difference				78.8
Confidence Limits (95%)				100 137	Confidence Limits (95%)				66 91

Group B15 Flank

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1257	1	358	293	81.8	1257	3	346	224	64.7
1259	3	407	645	158.5	1259	1	316	372	117.7
1260	1	317	530	167.2	1260	3	218	216	99.1
1261	3	276	372	134.8	1261	1	234	240	102.6
1263	3	320	457	142.8	1263	1	387	266	68.7
1264	1	288	299	103.8	1264	3	211	216	102.4
1265	1	301	330	109.6	1265	3	337	287	85.2
Mean Difference				128.4	Mean Difference				91.5
Confidence Limits (95%)				107 150	Confidence Limits (95%)				78 105

Group B15 Hip

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1257	2	319	465	145.8	1257	4	364	263	72.3
1259	4	434	405	93.3	1259	2	328	378	115.2
1260	2	334	549	164.4	1260	4	283	334	118.0
1261	4	294	335	113.9	1261	2	275	230	83.6
1263	4	400	504	126.0	1263	2	386	511	132.4
1264	2	274	311	113.5	1264	4	359	246	68.5
1265	2	406	439	108.1	1265	4	321	298	92.8
Mean Difference				123.6	Mean Difference				97.6
Confidence Limits (95%)				107 140	Confidence Limits (95%)				81 114

Group C3 Flank

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1585	1	347	344	99.1	1585	3	366	243	66.4
1590	3	406	317	78.1	1590	1	415	217	52.3
1591	1	404	337	83.4	1591	3	431	234	54.3
1587	1	379	281	74.1	1587	3	408	205	50.2
1586	3	412	329	79.9	1586	1	392	197	50.3
1598	1	338	385	113.9	1598	3	378	228	60.3
Mean Difference				88.1	Mean Difference				55.6
Confidence Limits (95%)				77 99	Confidence Limits (95%)				51 60

Group C3 Hip

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1585	2	451	416	92.2	1585	4	393	235	59.8
1590	4	433	360	83.1	1590	2	383	257	67.1
1591	2	393	266	67.7	1591	4	378	164	43.4
1587	2	404	324	80.2	1587	4	426	158	37.1
1586	4	422	547	129.6	1586	2	456	381	83.6
1598	2	428	238	55.6	1598	4	517	356	68.9
Mean Difference				84.7	Mean Difference				60.0
Confidence Limits (95%)				66 103	Confidence Limits (95%)				47 73

Group C4 Flank

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1350	1	404	208	51.5	1350	3	369	149	40.4
1351	3	385	274	71.2	1351	1	370	273	73.8
1352	1	401	149	37.2	1352	3	446	269	60.3
1353	3	421	316	75.1	1353	1	331	199	60.1
1357	1	429	414	96.5	1357	3	406	231	56.9
1358	3	350	311	88.9	1358	1	458	392	85.6
1359	1	385	332	86.2	1359	3	410	345	84.1
1360	3	402	364	90.5	1360	1	451	248	55.0
Mean Difference				74.6	Mean Difference				64.5
Confidence Limits (95%)				61 88	Confidence Limits (95%)				54 75

Group C4 Hip

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1350	2	467	266	57.0	1350	4	412	130	31.6
1351	4	400	306	76.5	1351	2	488	250	51.2
1352	2	480	353	73.5	1352	4	525	154	29.3
1353	4	390	255	65.4	1353	2	461	229	49.7
1357	2	473	363	76.7	1357	4	362	257	71.0
1358	4	350	312	89.1	1358	2	401	297	74.1
1359	2	420	355	84.5	1359	4	399	406	101.8
1360	4	392	361	92.1	1360	2	413	282	68.3
Mean Difference				76.9	Mean Difference				59.6
Confidence Limits (95%)				69 85	Confidence Limits (95%)				44 75

Group C5 Flank

	OPSITE				CONTROL			
Rat No.	Wd. No.	Wnd	Bx.	% Diff	Rat No.	Wd. No.	Wnd	Bx	% Diff
1616	1	406	461	113.5	1616	3	339	313	92.3
1621	3	358	401	112.0	1621	1	406	391	96.3
1618	1	299	359	120.1	1618	3	340	335	98.5
1620	3	312	287	92.0	1620	1	400	281	70.3
1617	1	232	234	100.9	1617	3	342	174	50.9
1569	3	339	368	108.6	1569	1	413	349	84.5
1619	1	401	481	120.0	1619	3	400	293	73.3
Mean Difference				109.6	Mean Difference				80.9
Confidence Limits (95%)				103 117	Confidence Limits (95%)				69 93

Group C5 Hip

	OPSITE				CONTROL			
Rat No.	Wd. No.	Wnd	Bx.	% Diff	Rat No.	Wd. No.	Wnd	Bx	% Diff
1616	2	382	402	105.2	1616	4	364	300	82.4
1621	4	367	385	104.9	1621	2	411	360	87.6
1618	2	403	490	121.6	1618	4	299	359	120.1
1620	4	446	257	57.6	1620	2	370	210	56.8
1617	2	384	405	105.5	1617	4	325	397	122.2
1569	4	385	379	98.4	1569	2	378	316	83.6
1619	2	370	331	89.5	1619	4	379	159	42.0
Mean Difference				97.5	Mean Difference				84.9
Confidence Limits (95%)				84 111	Confidence Limits (95%)				65 105

Group C10 Flank

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1309	1	328	573	174.7	1309	3	409	398	97.3
1310	3	388	513	132.2	1310	1	392	257	65.6
1311	1	391	545	139.4	1311	3	391	328	83.9
1312	3	377	397	105.3	1312	1	408	319	78.2
1313	1	348	352	101.1	1313	3	318	457	143.7
1315	1	431	477	110.7	1315	3	305	317	103.9
1316	3	416	509	122.4	1316	1	441	339	76.9
Mean Difference				126.5	Mean Difference				92.8
Confidence Limits (95%)				109 144	Confidence Limits (95%)				75 111

Group C10 Hip

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1309	2	377	536	142.2	1309	4	447	386	86.4
1310	4	366	511	139.6	1310	2	414	528	127.5
1311	2	381	537	140.9	1311	4	408	432	105.9
1312	4	378	460	121.7	1312	2	422	341	80.8
1313	2	354	499	141.0	1313	4	394	358	90.9
1315	2	414	471	113.8	1315	4	427	312	73.1
1316	4	514	488	94.9	1316	2	514	488	94.9
Mean Difference				127.7	Mean Difference				94.2
Confidence Limits (95%)				115 140	Confidence Limits (95%)				82 107

Group C15 Flank

		OPSITE				CONTROL			
Rat No.	Wd. No.	Wnd	Bx.	% Diff	Rat No.	Wd. No.	Wnd	Bx	% Diff
1268	1	300	301	100.3	1268	3	452	400	88.5
1269	1	386	441	114.2	1269	1	402	264	65.7
1270	1	341	353	103.5	1270	3	368	200	54.3
1271	1	308	466	151.3	1271	1	310	256	82.6
1272	3	287	439	153.0	1272	3	303	258	85.1
1274	3	275	468	170.2	1274	1	394	363	92.1
Mean Difference				132.1	Mean Difference				78.1
Confidence Limits (95%)				110 154	Confidence Limits (95%)				67 89

Group C15 Hip

		OPSITE				CONTROL			
Rat No.	Wd. No.	Wnd	Bx.	% Diff	Rat No.	Wd. No.	Wnd	Bx	% Diff
1268	2	389	458	117.7	1268	4	401	440	109.7
1269	2	315	490	155.6	1269	4	426	358	84.0
1270	2	446	328	73.5	1270	4	350	198	56.6
1271	2	308	466	151.3	1271	4	331	277	83.7
1272	4	288	439	152.4	1272	2	296	351	118.6
1274	4	314	398	126.8	1274	2	295	324	109.8
Mean Difference				129.6	Mean Difference				93.7
Confidence Limits (95%)				107 153	Confidence Limits (95%)				77 111

Group D4 Flank

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
361	1	350	311	88.9	361	3	382	214	56.0
362	3	391	402	102.8	362	1	379	334	88.1
363	1	400	150	37.5	363	3	371	245	66.0
364	3	385	196	50.9	364	1	395	125	31.6
365	1	369	317	85.9	365	3	361	171	47.4
366	3	322	284	88.2	366	1	395	269	68.1
367	1	374	317	84.8	367	3	395	140	35.4
368	3	322	365	113.4	368	1	395	180	45.6
Mean Difference				81.5	Mean Difference				54.8
Confidence Limits (95%)				65 98	Confidence Limits (95%)				43 67

Group D4 Hip

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
361	2	367	297	80.9	361	4	333	154	46.2
362	4	365	375	102.7	362	2	382	321	84.0
363	2	365	202	55.3	363	4	389	284	73.0
364	4	384	307	79.9	364	2	378	182	48.1
365	2	393	311	79.1	365	4	379	269	71.0
366	4	405	390	96.3	366	2	411	256	62.3
367	2	382	454	118.8	367	4	387	268	69.3
368	4	401	459	114.5	368	2	415	262	63.1
Mean Difference				91.0	Mean Difference				64.6
Confidence Limits (95%)				77 105	Confidence Limits (95%)				56 73

Group D5 Flank

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1532	1	399	362	90.7	1532	3	387	287	74.2
1533	3	364	322	88.5	1533	1	434	351	80.9
1534	1	419	400	95.5	1534	3	393	324	82.4
1535	3	416	373	89.7	1535	1	374	250	66.8
1536	1	358	317	88.5	1536	3	335	247	73.7
1537	3	413	390	94.4	1537	1	434	357	82.3
1539	1	437	423	96.8	1539	3	415	333	80.2
1540	3	481	459	95.4	1540	1	414	322	77.8
Mean Difference				92.4	Mean Difference				77.3
Confidence Limits (95%)				90 95	Confidence Limits (95%)				74 81

Group D5 Hip

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1532	2	418	370	88.5	1532	4	385	250	64.9
1533	4	364	322	88.5	1533	2	452	310	68.6
1534	2	391	387	99.0	1534	4	381	300	78.7
1535	4	343	250	72.9	1535	2	443	313	70.7
1536	2	431	350	81.2	1536	4	395	256	64.8
1537	4	387	350	90.4	1537	2	415	350	84.3
1539	2	407	373	91.6	1539	4	392	301	76.8
1540	4	427	389	91.1	1540	2	422	331	78.4
Mean Difference				87.9	Mean Difference				73.4
Confidence Limits (95%)				83 93	Confidence Limits (95%)				69 78

Group D10 Flank

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1318	1	408	555	136.1	1318	3	387	463	119.6
1319	3	402	509	126.5	1319	1	373	250	67.0
1320	1	443	459	103.6	1320	3	381	231	60.6
1321	3	417	437	104.8	1321	1	446	293	65.7
1322	1	430	488	113.5	1322	3	403	294	73.0
1323	3	426	456	107.0	1323	1	522	365	69.9
1324	1	404	371	91.8	1324	3	393	300	76.3
1325	3	389	473	121.6	1325	1	367	312	85.0
Mean Difference				113.1	Mean Difference				77.2
Confidence Limits (95%)				104 122	Confidence Limits (95%)				65 89

Group D10 Hip

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1318	2	424	531	125.2	1318	4	418	360	86.1
1319	4	378	400	105.8	1319	2	386	348	90.2
1320	2	443	459	103.6	1320	4	373	247	66.2
1321	4	350	430	122.9	1321	2	361	296	82.0
1322	2	371	438	118.1	1322	4	457	356	77.9
1323	4	436	452	103.7	1323	2	436	322	73.9
1324	2	448	403	90.0	1324	4	429	207	48.3
1325	4	450	464	103.1	1325	2	482	317	65.8
Mean Difference				109.0	Mean Difference				73.8
Confidence Limits (95%)				101 117	Confidence Limits (95%)				65 83

Group D15 Flank

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1278	1	359	400	111.4	1278	3	401	364	90.8
1279	3	368	392	106.5	1279	1	379	337	88.9
1275	1	376	370	98.4	1275	3	412	390	94.7
1281	3	395	410	103.8	1281	1	383	301	78.6
1282	1	361	397	110.0	1282	3	403	365	90.6
1284	3	387	395	102.1	1284	1	394	379	96.2
1276	1	423	450	106.4	1276	3	379	325	85.8
Mean Difference				105.5	Mean Difference				89.4
Confidence Limits (95%)				102 109	Confidence Limits (95%)				85 93

Group D15 Hip

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1278	2	374	385	102.9	1278	4	423	437	103.3
1279	4	401	429	107.0	1279	2	389	301	77.4
1275	2	334	341	102.1	1275	4	379	350	92.3
1281	4	374	380	101.6	1281	2	423	358	84.6
1282	2	387	412	106.5	1282	4	382	331	86.6
1284	4	385	390	101.3	1284	2	376	381	101.3
1276	2	417	417	100.0	1276	4	390	359	92.1
Mean Difference				103.1	Mean Difference				91.1
Confidence Limits (95%)				101 105	Confidence Limits (95%)				85 97

Group E5 Flank

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1544	3	391	302	77.2	1544	1	299	248	82.9
1548	3	303	282	93.1	1548	1	387	196	50.6
1549	1	418	301	72.0	1549	3	391	201	51.4
1550	3	304	337	110.9	1550	1	418	203	48.6
1541	1	453	401	88.5	1541	3	328	209	63.7
1564	3	370	367	99.2	1564	1	259	213	82.2
1556	3	399	368	92.2	1556	1	380	213	56.1
Mean Difference				90.4	Mean Difference				62.2
Confidence Limits (95%)				81 99	Confidence Limits (95%)				52 72

Group E5 Hip

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1544	4	366	302	82.5	1544	2	355	246	69.3
1548	4	384	351	91.4	1548	2	371	217	58.5
1549	2	407	407	100.0	1549	4	403	260	64.5
1550	4	413	391	94.7	1550	2	389	218	56.0
1541	2	445	383	86.1	1541	4	320	235	73.4
1564	4	412	441	107.0	1564	2	403	222	55.1
1556	4	490	441	90.0	1556	2	425	222	52.2
Mean Difference				93.1	Mean Difference				61.3
Confidence Limits (95%)				87 99	Confidence Limits (95%)				56 67

Group E10 Flank

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1326	1	379	402	106.1	1326	3	346	344	99.4
1327	3	429	539	125.6	1327	1	361	352	97.5
1328	1	399	418	104.8	1328	3	382	308	80.6
1329	3	481	340	70.7	1329	1	384	227	59.1
1330	1	346	402	116.2	1330	3	395	301	76.2
1332	1	430	337	78.4	1332	3	404	275	68.1
1333	3	410	360	87.8	1333	1	435	258	59.3
Mean Difference				98.5	Mean Difference				77.2
Confidence Limits (95%)				85 112	Confidence Limits (95%)				66 89

Group E10 Hip

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1326	4	460	432	93.9	1326	2	445	293	65.8
1327	4	328	493	150.3	1327	2	377	421	111.7
1328	2	433	425	98.2	1328	4	474	339	71.5
1329	4	412	268	65.0	1329	2	340	276	81.2
1330	2	388	415	107.0	1330	4	426	350	82.2
1332	4	474	398	84.0	1332	2	503	302	60.0
1333	4	446	297	66.6	1333	2	475	327	68.8
Mean Difference				95.0	Mean Difference				77.3
Confidence Limits (95%)				75 115	Confidence Limits (95%)				66 89

Group E15 Flank

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1324	3	401	455	113.5	1324	3	290	220	75.9
1287	1	326	400	122.7	1287	1	273	303	111.0
1288	3	326	472	144.8	1288	3	320	315	98.4
1289	1	392	437	111.5	1289	1	352	302	85.8
1290	1	398	368	92.5	1290	3	327	291	89.0
1291	1	360	470	130.6	1291	1	321	328	102.2
1292	3	322	395	122.7	1292	3	260	231	88.8
1317	1	395	539	136.5	1317	1	367	263	71.7
Mean Difference				121.8	Mean Difference				90.3
Confidence Limits (95%)				111 132	Confidence Limits (95%)				82 99

Group E15 Hip

Rat No.	OPSITE Wd. No.	Wnd	Bx.	% Diff	Rat No.	CONTROL Wd. No.	Wnd	Bx	% Diff
1324	2	420	504	120.0	1324	4	379	344	90.8
1287	4	358	411	114.8	1287	2	393	270	68.7
1288	2	414	442	106.8	1288	4	350	327	93.4
1289	4	330	360	109.1	1289	2	310	225	72.6
1290	2	317	320	100.9	1290	4	358	293	81.8
1291	4	380	433	113.9	1291	2	285	336	117.9
1292	2	335	387	115.5	1292	4	415	295	71.1
1317	4	350	437	124.9	1317	2	360	266	73.9
Mean Difference				113.2	Mean Difference				83.8
Confidence Limits (95%)				108 118	Confidence Limits (95%)				73 95

Statistical Analysis

A paired Student "t" test comparing Flank and Hip Surface areas

Flank Wounds

Groups	Deg. Freed.	t	p	Mean M.Diff	95% Confidence Limits	
A2	6	1.494	0.1858	21.643	-6.51	49.80
A3	7	2.531	0.0390	20.613	5.18	36.04
A4	7	4.035	0.0050	30.850	16.37	45.33
A5	5	3.916	0.0112	33.517	16.27	50.76
A10	7	5.621	0.0008	48.538	32.18	64.90
A15	6	3.775	0.0094	71.657	34.58	108.74
B2	6	0.332	0.7510	2.729	-13.23	18.69
B3	5	4.216	0.0084	20.386	10.64	30.13
B4	6	3.213	0.0183	24.357	9.63	39.09
B5	6	2.871	0.0284	24.866	8.04	41.73
B10	6	4.534	0.0040	31.271	17.87	44.67
B15	6	3.686	0.0103	36.871	17.43	56.31
C3	6	8.549	0.0001	33.743	26.07	41.41
C4	7	1.402	0.2037	10.112	-3.55	23.78
C5	6	5.552	0.0014	28.714	18.66	38.76
C10	6	2.167	0.0734	33.757	3.48	64.03
C15	5	5.569	0.0026	54.033	34.48	73.58
D4	7	2.661	0.0324	26.775	7.71	45.84
D5	7	9.561	0.0000	15.150	12.15	18.15
D10	7	7.111	0.0002	35.975	26.39	45.56
D15	6	5.269	0.0019	16.143	10.19	22.10
E5	6	3.474	0.0132	28.229	12.44	44.02
E10	6	4.651	0.0035	21.343	12.43	30.26
E15	7	4.629	0.0024	31.500	18.61	44.39

Hip Wounds

A2	6	1.894	0.1070	14.743	-0.38	29.87
A3	7	3.758	0.0071	14.913	7.39	22.43
A4	7	4.608	0.0025	29.463	17.35	41.58
A5	5	4.154	0.0089	13.483	6.94	20.02
A10	7	2.791	0.0269	28.288	9.08	47.49
A15	5	2.002	0.1017	30.283	-0.20	60.77
B2	7	2.041	0.0806	18.963	1.36	36.56
B3	5	6.099	0.0017	30.967	20.74	41.20
B4	6	4.335	0.0048	20.014	11.08	28.94
B5	5	5.930	0.0019	34.983	23.10	46.87
B10	6	5.806	0.0011	39.586	26.34	52.83
B15	6	2.091	0.8150	26.029	1.84	50.22
C3	5	2.784	0.0387	24.750	6.83	42.67
C4	7	2.727	0.0295	17.225	5.26	29.19
C5	6	1.639	0.1523	12.571	-2.33	27.47
C10	6	4.377	0.0047	33.514	18.64	48.39
C15	5	3.191	0.0242	35.817	13.20	58.44
D4	7	3.258	0.0139	26.313	11.01	41.62
D5	7	5.608	0.0008	14.500	9.60	19.40
D10	7	11.387	0.0000	35.250	29.38	41.12
D15	6	2.898	0.0274	11.971	3.95	20.00
E5	6	5.919	0.0010	31.814	21.37	42.26
E10	6	2.410	0.0526	17.686	3.43	31.94
E15	7	4.411	0.0031	29.463	16.81	42.12

Flank Wounds
The differences between the Opsite and the Control wounds at
the point of maximal contraction were collected to produce the following data.

Mean Difference	26.2%
Std Deviation	4.31
Std. Error of the Mean	2.15
95% Confidence Limits	20.22 32.18

Flank Wound v Hip Wound

This table lists the results of the paired Student's "t" tests comparing the differences between the Opsite and the control wounds on the flank and hip areas. If the hip wound is a true model of a flexor crease there should be a significant difference between both sets of figures.

	^{0}F	t	p	Mean Diff.	95% Confidence Limits	
A1						
A2	6	0.406	0.6990	6.943	-34.93	48.82
A3	7	0.575	0.5832	5.687	-17.69	29.07
A4	7	0.180	0.8619	1.387	-16.80	19.57
A5	5	2.374	0.0636	20.017	-1.66	41.69
A10	7	1.334	0.2240	20.213	15.62	56.04
A15	5	1.448	0.2074	48.150	-37.35	133.65
B2	6	-1.480	0.1894	-13.014	-34.53	8.50
B3	5	-5.266	0.0033	-10.567	-15.72	-5.41
B4	6	0.533	0.6001	4.343	-14.87	23.55
B5	5	-1.425	0.2134	-14.383	-40.32	11.56
B10	6	-0.828	0.4392	-8.300	-32.82	16.22
B15	6	0.570	0.5892	10.875	35.73	57.45
C3	5	0.620	0.5732	7.638	-25.11	40.47
C4	7	-0.643	0.5405	-7.150	-33.43	19.13
C5	6	1.751	0.1305	16.144	-6.40	38.63
C10	6	0.012	0.9910	0.229	-47.34	47.80
C15	5	1.492	0.1960	18.217	-13.18	49.61
D4	7	0.078	0.9402	0.425	-12.10	13.36
D5	7	0.179	0.8613	0.650	-7.94	9.24
D10	7	0.093	0.9289	0.700	-17.19	18.59
D15	6	0.987	0.3619	4.200	-6.22	14.62
E5	6	-0.462	0.6600	-3.586	-22.56	15.39
E10	6	0.463	0.6595	3.657	-15.66	22.97
E15	7	0.238	0.8190	2.013	-18.01	22.04

The significance of these figures is discussed in the text.

Myofibroblast Results

gp	Rat No	Bx Tme (weeks)	MEAN NUMBERS OF MFBS PER UNIT SURFACE AREA Flank Opsite	Flank Control	Hip Opsite	Hip Control
A1	565	1	3	10	5	10
	566	1	2	12	3	8
	567	1	5	11	4	12
	568	1	3	14	6	8
	570	1	3	8	3	6
	573	1	2	13	3	9
	574	1	1	9	3	12
	575	1	8	9	4	11
	Mean		3	11	4	10
	Confidence Limits (95%)		2 5	9 12	3 5	8 11
A2	372	2	5	9	8	14
	376	2	3	8	7	12
	381	2	6	8	7	14
	388	2	1	12	5	14
	397	2	2	9	5	19
	392	2	3	6	9	12
	373	2	3	7	3	13
	Mean		3	8	6	14
	Confidence Limits (95%)		2 4	7 10	5 8	12 16
A3	341	3	11	21	15	30
	340	3	9	26	16	17
	339	3	9	23	18	21
	338	3	13	20	6	25
	337	3	10	20	9	26
	336	3	11	19	14	25
	335	3	12	23	12	25
	334	3	8	22	12	27
	Mean		10	22	13	25
	Confidence Limits (95%)		9 11	20 23	10 15	22 27

gp	Rat No	Bx Tme (weeks)	MEAN NUMBERS OF MFBS PER UNIT SURFACE AREA Flank Opsite	Flank Control	Hip Opsite	Hip Control
A4	552	4	9	12	9	16
	557	4	11	13	12	15
	559	4	10	13	12	15
	558	4	10	10	13	12
	561	4	12	11	16	18
	560	4	4	19	8	17
	562	4	13	16	13	13
	571	4	8	15	14	15
	Mean		10	14	12	15
	Confidence Limits (95%)		8 11	12 16	10 14	14 16
A5	495	5	2	10	12	17
	498	5	2	13	13	18
	500	5	5	12	14	16
	510	5	6	8	11	12
	512	5	4	9	10	9
	511	5	3	11	11	19
	515	5	3	10	16	20
	Mean		4	10	12	16
	Confidence Limits (95%)		3 5	9 12	11 14	13 19
A10	293	10	2	5	4	8
	294	10	2	11	3	9
	295	10	1	4	9	1
	296	10	0	4	6	4
	297	10	6	12	4	5
	298	10	3	6	4	6
	299	10	4	6	3	8
	300	10	2	3	1	7
	Mean		3	6	4	6
	Confidence Limits (95%)		1 4	4 9	3 6	4 8
A15	249	15	0	4	6	7
	250	15	0	3	4	8
	251	15	1	3	9	2
	252	15	2	1	9	5
	253	15	2	8	2	6
	255	15	2	5	3	4
	256	15	1	5	7	9
	Mean		1	4	6	6
	Confidence Limits (95%)		1 2	3 6	4 8	4 8

gp	Rat No	Bx Tme (weeks)	MEAN NUMBERS OF MFBS PER UNIT SURFACE AREA Flank Opsite	Flank Control	Hip Opsite	Hip Control
B2	600	2	6	10	7	12
	601		7	12	7	11
	602		4	10	9	12
	603		5	14	10	10
	606		2	14	10	9
	605		9	13	9	12
	604		6	11	7	6
	511		6	13	7	13
	Mean		6	12	8	11
	Confidence Limits (95%)		4 7	11 13	7 9	9 12
B3	572	3	10	20	8	19
	578		12	18	8	20
	581		9	15	7	22
	582		7	17	9	23
	583		9	17	8	22
	584		6	16	8	18
	Mean		9	17	8	21
	Confidence Limits (95%)		7 10	16 18	8 8	19 22
B4	343	4	11	17	14	21
	344		10	16	9	19
	345		9	21	22	19
	346		13	19	7	22
	347		12	18	6	18
	348		14	16	10	22
	349		10	17	13	19
	Mean		11	18	12	20
	Confidence Limits (95%)		10 13	16 19	8 15	19 21

gp	Rat No	Bx Tme (weeks)	MEAN NUMBERS OF MFBS PER UNIT SURFACE AREA Flank Opsite	Flank Control	Hip Opsite	Hip Control
B5	524	5	0	9	7	10
	610		2	12	8	10
	611		5	13	8	9
	614		9	13	8	8
	608		4	10	9	8
	613		3	11	6	11
	615		1	12	6	11
	Mean		3	11	7	10
	Confidence Limits (95%)		1 5	10 12	7 8	9 10
B10	302	10	0	7	6	10
	303		1	7	1	10
	304		2	9	12	11
	305		4	10	12	7
	306		3	8	9	9
	307		3	11	8	9
	308		2	9	7	6
	Mean		2	9	8	9
	Confidence Limits (95%)		1 3	8 10	5 10	8 10
B15	257	15	1	6	7	2
	259		0	7	9	7
	260		1	5	10	12
	261		2	6	7	11
	263		1	6	9	9
	264		1	7	9	10
	265		2	5	9	11
	Mean		1	6	9	9
	Confidence Limits (95%)		1 2	5 7	8 9	7 11

gp	Rat No	Bx Tme (weeks)	MEAN NUMBERS OF MFBS PER UNIT SURFACE AREA Flank Opsite	Flank Control	Hip Opsite	Hip Control
C3	585	3	11	13	12	22
	590		9	13	12	23
	591		10	15	11	23
	596		9	17	13	20
	586		9	14	12	21
	587		10	14	13	19
	598		11	12	13	17
	Mean		10	14	12	21
	Confidence Limits (95%)		9	13	12	19
			10	15	13	22
C4	350	4	15	22	17	25
	351		15	21	12	23
	352		9	21	17	21
	352		12	21	15	21
	357		14	19	15	21
	358		16	19	19	18
	359		10	17	12	21
	360		10	15	19	24
	Mean		13	19	16	22
	Confidence Limits (95%)		11	18	14	20
			14	21	18	23

gp	Rat No	Bx Tme (weeks)	MEAN NUMBERS OF MFBS PER UNIT SURFACE AREA Flank Opsite	Flank Control	Hip Opsite	Hip Control
C5	616	5	5	17	10	12
	621		5	17	10	13
	618		4	15	11	10
	620		4	12	4	11
	617		2	9	8	14
	569		2	11	9	7
	619		6	10	2	9
	Mean		4	13	8	11
	Confidence Limits (95%)		3	11	5	9
			5	15	10	13
C10	309	10	2	5	4	10
	310		2	5	7	7
	311		1	1	6	8
	312		1	2	6	6
	313		1	6	6	7
	315		2	7	5	7
	316		1	6	5	7
	Mean		1	5	6	7
	Confidence Limits (95%)		1	3	5	7
			2	6	6	8
C15	268	15	1	4	6	6
	269		1	3	6	7
	270		2	4	5	5
	271		1	5	5	7
	272		2	7	7	8
	274		1	4	2	4
	Mean		1	5	5	6
	Confidence Limits (95%)		1	3	4	5
			2	6	6	7

gp	Rat No	Bx Tme (weeks)	MEAN NUMBERS OF MFBS PER UNIT SURFACE AREA Flank Opsite	Flank Control	Hip Opsite	Hip Control
D4	361	4	16	25	19	22
	362		16	25	19	22
	363		17	20	20	27
	364		12	13	15	27
	365		22	19	15	18
	366		19	22	17	27
	367		17	21	11	25
	368		15	21	19	18
	Mean		17	21	17	23
	Confidence Limits (95%)		15	18	15	21
			19	23	19	26
D5	532	5	9	15	11	7
	533		9	17	13	17
	534		8	13	13	13
	535		8	15	9	13
	537		8	15	9	14
	539		7	11	15	11
	540		5	20	15	19
	Mean		8	15	12	13
	Confidence Limits (95%)		7	13	10	11
			9	17	14	16
D10	318	10	3	7	10	11
	319		3	7	4	19
	320		3	8	6	13
	321		1	8	7	10
	322		1	8	10	10
	323		1	8	9	9
	324		1	8	7	3
	325		3	7	8	12
	Mean		2	8	8	11
	Confidence Limits (95%)		1	7	6	8
			3	8	9	14
D15	278	15	1	5	2	11
	279		1	9	9	10
	275		1	3	9	7
	281		4	3	7	7
	282		1	6	7	7
	284		3	6	8	7
	276		2	7	7	7
	Mean		2	6	7	8
	Confidence Limits (95%)		1	4	5	7
			3	7	9	9

gp	Rat No	Bx Tme (weeks)	MEAN NUMBERS OF MFBS PER UNIT SURFACE AREA Flank Opsite	Flank Control	Hip Opsite	Hip Control
E5	544	5	7	10	9	10
	548		4	10	9	13
	549		4	9	8	13
	550		3	9	11	12
	541		1	14	10	9
	556		5	7	9	11
	Mean		4	10	9	11
	Confidence Limits (95%)		3 5	8 12	9 10	10 13
E10	326	10	1	7	6	12
	327		1	5	10	12
	328		1	7	7	13
	329		2	4	9	9
	330		5	4	8	11
	332		1	9	4	7
	333		1	7	2	6
	Mean		2	6	7	10
	Confidence Limits (95%)		1 3	5 7	5 9	8 12
E15	324	15	1	3	9	12
	287		0	3	9	7
	288		0	1	9	10
	289		2	4	7	6
	290		3	1	7	4
	291		3	3	5	6
	292		2	1	3	6
	317		3	4	3	3
	Mean		2	3	7	7
	Confidence Limits (95%)		1 3	2 3	5 8	5 9

Statistical Comparison of Myofibroblast Numbers under the Control and the Opsite Skin Grafts

Groups were compared using the paired Student's "t" test.

Flank Wounds

	Degrees of Freedom	t	p	Mean Diff.	95% Confidence Limits	
A1	7	6.009	0.0005	7.357	4.52	10.23
A2	6	4.500	0.0041	5.143	2.35	7.94
A3	7	9.524	0.0000	11.375	8.55	14.20
A4	7	2.244	0.0598	4.000	-0.22	8.22
A5	6	6.492	0.0006	6.857	4.27	9.44
A10	7	4.329	0.0034	3.875	1.76	5.99
A15	6	3.674	0.0104	3.000	1.00	5.00
B2	7	6.619	0.0003	6.500	4.18	8.82
B3	5	10.38	0.0001	8.333	6.27	10.40
B4	6	5.793	0.0012	6.429	3.71	9.14
B5	6	8.892	0.0001	8.000	5.80	10.20
B10	6	17.81	0.0000	6.571	5.67	7.47
B15	6	9.553	0.0001	4.875	3.61	6.10
C3	6	4.833	0.0029	4.143	2.05	6.24
C4	7	6.906	0.0002	6.750	4.44	9.06
C5	6	8.088	0.0002	9.000	6.28	11.72
C10	6	4.085	0.0065	3.143	1.26	5.03
C15	5	6.635	0.0012	3.167	1.94	4.39
D4	7	2.804	0.0264	4.000	0.63	7.37
D5	6	5.461	0.0016	7.429	4.10	10.76
D10	7	10.56	0.0000	5.625	4.37	6.88
D15	6	3.495	0.0129	3.714	1.11	6.31
E5	5	3.693	0.0141	5.833	1.77	9.89
E10	6	3.845	0.0085	4.429	1.61	7.25
E15	7	1.271	0.2443	0.750	-0.65	2.15

Hip Wounds

A1	7	6.666	0.0003	5.6250	3.63	7.62
A2	6	5.610	0.0014	7.714	4.35	11.48
A3	7	5.115	0.0014	11.750	6.32	17.18
A4	7	2.479	0.0423	3.000	0.14	5.86
A5	6	3.023	0.0230	3.429	0.66	6.20
A10	7	1.030	0.3371	1.750	-2.27	5.77
A15	6	0.091	0.9302	0.143	-3.69	3.97
B2	7	2.468	0.0430	2.375	0.10	4.65
B3	5	15.779	0.0000	12.667	10.60	14.73
B4	6	3.772	0.0093	8.429	2.96	13.90
B5	6	2.423	0.0517	2.143	-0.02	4.31
B10	6	0.579	0.5726	1.000	-3.10	5.10
B15	6	0.253	0.8085	0.268	-2.47	3.05
C3	6	7.747	0.0002	8.429	5.77	11.09
C4	7	4.681	0.0023	6.000	2.97	9.03
C5	6	2.238	0.0666	3.143	-0.29	6.58
C10	6	2.414	0.0523	1.875	-0.03	3.74
C15	5	2.739	0.0409	1.000	0.06	1.94
D4	7	3.444	0.0108	6.375	2.00	10.75
D5	6	0.862	0.4218	1.286	-2.36	4.94
D10	7	1.598	0.1540	3.250	-1.56	8.06
D15	6	0.725	0.4960	1.000	-2.38	4.38
E5	5	2.236	0.0756	2.000	-0.30	4.30
E10	6	4.221	0.0056	3.429	1.44	5.42
E15	7	0.323	0.7650	0.250	-1.58	2.08

Appendix 4

Human Data

Donor Site Study

CONVENTIONAL DRESSING				SYNTHETIC DRESSING			
Dressing No.	day 0 (cm2)	day 15 (cm2)	% diff	Dressing No.	day 0 (cm2)	day 17 (cm2)	% diff
1	80.12	65.22	18.60	26	66.22	56.22	15.10
2	84.36	69.35	17.79	27	98.71	89.35	9.48
3	89.51	80.23	10.37	28	97.33	91.28	6.22
4	90.22	64.36	28.66	29	78.22	75.09	4.00
5	78.35	62.00	20.87	30	84.79	78.33	7.62
6	67.56	54.37	19.52	31	85.33	80.55	5.60
7	83.21	65.39	21.42	32	98.01	88.21	10.00
8	89.78	77.01	14.22	33	63.58	62.80	1.23
9	79.39	67.23	15.32	34	58.09	55.29	4.82
10	99.01	89.51	9.59	35	81.66	78.44	3.94
11	100.15	75.21	24.90	36	89.08	78.09	12.34
12	78.65	64.02	18.60	37	75.38	70.00	7.14
13	67.21	55.38	17.60	38	78.21	73.66	5.82
14	90.36	77.09	14.69	39	88.59	75.98	14.23
15	98.81	84.10	14.89	40	99.33	91.56	7.82
16	95.64	57.38	40.00	41	56.05	50.34	10.19
17	80.64	59.71	25.95	42	74.59	66.01	11.50
18	81.48	60.09	26.25	43	81.66	74.57	8.68
19	87.39	77.53	11.28	44	85.97	76.76	10.71
20	84.67	68.11	19.56	45	69.45	65.94	5.05
21	69.20	50.39	27.18	46	85.11	74.81	12.10
22	90.57	71.58	20.97	47	64.77	60.33	6.86
23	81.57	65.98	19.11	48	89.32	84.69	5.18
24	89.37	69.44	22.30	49	99.45	89.11	10.40
25	78.00	65.05	16.60	50	94.67	90.00	4.93
mean	84.69	67.88	19.85		80.41	73.84	8.04
95% Conf. Lim.	81.19 88.18	64.21 71.55	17.31 22.39		75.37 85.46	69.26 78.42	6.70 9.38

Statistical Analysis

Unpaired t test comparing initial wound size both groups

	Conventional Donor Site	Occlusive Donor Site
Mean	84.61	81.74

assume variance equal

t =	0.897
p =	0.3743
95% cl	-3.56
	9.29

assume variance unequal

d =	0.897
p =	0.3697
95%cl	-3.40
	9.13

This indicates that the null hypothesis stating that there is a difference between the initial sizes of both groups is not proven.

Unpaired t test comparing the contraction differences of both Groups

	Conventional Dressing	Occlusive Dressing
Mean	19.85	8.04

assume variance equal

t =	7.91
p <	0.00
95% cl	8.81
	14.82

assume variance unequal

d =	7.91
p <	0.00
95%cl	8.88
	14.74

The hypothesis stating that there is a difference between dressing groups can be proved.

Skin Graft Study

All measurements are in square millimeters

Patient Code	Size at Wounding		Size at 1 Mth		Size at 6 Mnths	
	Control	Opsite	Control	Opsite	Control	Opsite
MB	8614	9863	7347	7721	4307	6115
LH1	1719	1975	1264	1368	859	1580
LH2	2265	3489	1870	2699	1271	2975
WJ	6470	4752	5662	4550	3558	3944
VO	6857	6442	4700	5080	4114	5417
SR	4072	3349	2850	2511	3123	2819
VS	1710	3318	1641	2564	923	2388
JM	3678	4070	2280	3418	1655	2017
PM	11920	6835	8940	5399	6198	6021
CM	4078	963	3592	595	3437	870
LM	10622	1965	9065	1806	6752	1627
CB	1547	3858	1178	3120	1105	2960
mean	5296	4239	4199	3402	3108	3227
95% Confidence Limits	3016	2673	2341	2140	1824	2098
	7575	5807	6056	4664	4392	4357

Patient Code	Size at 12 Mths	
	Control	Opsite
MB	5168	9342
LH1	1021	1817
LH2	1342	3140
WJ	4076	4514
VO	4251	6171
SR	3361	2913
VS	1011	2789
JM	2280	2753
PM	6230	6235
CM	3652	932
LM	7011	1760
CB	1213	3327
mean	3384	3807
95% Confidence Limits	2070	2291
	4698	5324

Paired t test comparing the size of the initial wounds

t =	-1.147
p =	0.276
md	-1056
95% cl	-3083
cl	971

The difference between the initial wound sizes is not significant.

Skin Graft Study

Wound Contraction Data

Percentage Contraction

C = Control Wounds
O = Opsite Wounds

Patient Code	1 Month		6 Months		12 Months	
	C	O	C	O	C	O
MB	85.291	78.282	50.000	61.999	59.995	94.718
LH1	73.531	69.266	49.971	80.000	59.395	92.000
LH2	82.561	77.357	56.115	85.268	59.249	89.997
WJ	87.512	95.749	54.992	82.997	62.998	94.992
VO	68.543	78.857	59.997	84.089	61.995	95.793
SR	69.990	74.978	76.694	84.174	82.539	86.981
VS	95.965	77.275	53.977	71.971	59.123	84.057
JM	61.990	83.980	44.997	49.558	61.990	67.641
PM	75.000	78.990	51.997	88.091	52.265	91.222
CM	88.082	61.786	84.282	90.343	89.554	96.781
LM	85.342	91.908	63.566	82.799	66.005	89.567
CB	76.147	80.871	71.429	76.724	78.410	86.236

The above data columns list wound size expressed as a percentage of the original surface area

Paired t tests on Data columns

	1 mth	6 mths	12 mths
t =	-0.014	5.701	6.121
p =	0.9888	0.0001	0.0001
md	-0.055	18.330	23.039
cl	-8.40	11.25	14.75
cl	8.29	25.41	31.32

There is no significant difference between wound sizes at 1 month.
Wound size is significantly different at 6 and 12 months.

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Eur J Plast Surg (1990) 13:189–191

European Journal of Plastic Surgery

Originals

Reduction of open wound contraction in humans with a synthetic dressing

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Summary. Many articles have been written about wound contraction. The majority of these use animals as these experiments are impossible to repeat in humans for ethical reasons. Skin graft donor sites have been used in this study as repeatable controlled wounds. Wound contraction does occur in these wounds and it can be reduced by covering the wound with a synthetic dressing.

Key words: Skin graft donor sites – Contraction – Synthetic dressings

Covering a wound with a full thickness skin graft effectively stops it contracting [1, 7, 11, 13]. Animal studies have shown that, in the short term, synthetic dressings have the same effect on reducing wound contraction as full thickness skin grafts [4, 5]. A study was therefore set up to see if a synthetic dressing had the same effect on open wounds in humans. If this were the case then the temporary dressing of burn wounds with synthetic dressings would reduce the stimulus to wound contraction which would perphaps reduce the need for long-term physiotherapy, splintage therapy and possibly the need for secondary surgery.

Split skin graft donor sites were used as wound models in this study as they are made in a repeatable controlled procedure, can be treated in a similar manner, and healing is independent of the primary defect for which the skin graft is being used. The dressing used in this study (Transigen, Smith and Nephew) is a synthetic wound dressing designed to allow excess moisture to evaporate in a controlled manner ensuring the wound is always kept moist. It is a soft pliable dressing that does not adhere to moist surfaces.

Excess fluid from the wound surface passes through pores in the inner membrane to accumulate and then evaporate through the outer membrane thus allowing wound healing to take place in a moist environment without excessive fluid accumulating under the dressing.

Materials and methods

Fifty-five donor sites on 55 patients were studied. All donor sites were on the thigh as large donor sites (at least 10×5 cm) were used to minimize experimental error. Patients with more than one donor site per thigh were excluded so that all wounds studied were surrounded by normal skin.

Donor site size was assessed in theatre by compressing a sterile paper towel on to the area after allowing capillary bleeding to take place. The mark made was carefully cut out and re-applied to ensure accuracy. An acetate sheet tracing was later taken and the initial towel discarded.

The dressing regimen was then chosen by a randomizing technique allocating the donor site to be dressed conventionally or with Transigen.

Conventional dressing consisted of covering the wound with paraffin impregnated gauze then dressing swabs covered by cotton wool held in place with a crepe bandage.

The transigen dressing regimen consisted of drying the surrounding skin with a gauze swab and then applying the dressing and loosely covering this with a crepe bandage.

Both dressings were left undisturbed for two weeks. The dressings were taken down in either the outpatient or ward environment by the nursing staff. Tracings were then taken by one of the authors of the epithelialised areas without him knowing which dressing had been used. The surface areas were then calculated and compared.

Results

Infection occurred in two donor sites covered with Transigen and three conventionally dressed donor sites. These were excluded from the study and responded to local dressing treatment.

The composition of both groups was similar with regards to age and sex (Table 1).

The mean time from graft harvesting to dressing removal was 15.3 days (standard error of the mean 1.9 days) in the conventionally dressed group and 17 days (standard error of the mean 1.5 days) in the Transigen group.

Mean wound contraction was 20% in the conventionally treated wound and 8% in the Transigen dressed

Table 1. See text

	Number of patients	Age range	Mean age
Male (C)	10	39–61	49.5
Female (C)	15	50–67	59
Male (T)	14	43–64	53
Female (T)	11	45–69	59.4

C = Conventional dressing
T = Transigen dressing

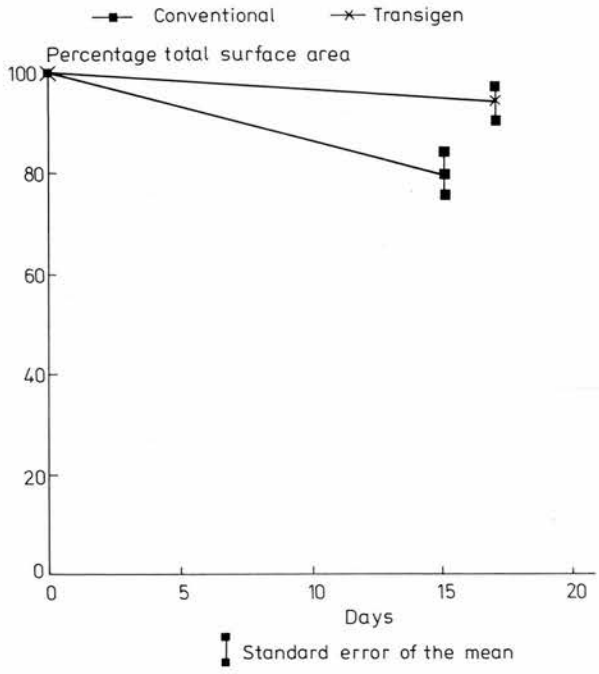

Fig. 1. Donor site contraction when dressings are removed

group (Fig. 1). This difference was statistically significant ($p < 0.04$).

Discussion

We did not measure the graft thickness and biopsy the underlying dermis to ensure that all wounds were similar. The study was designed so that:

1) skin graft harvesting was carried out before dressing groups were allocated.
2) measurements were taken after dressing removal without knowing which dressing had been used.

Therefore, with the random allocation of dressings, and measurements being taken without knowing which dressing was used, it would seem most likely that the composition of both groups would be stastically similar with regards to donor site depth [8].

Frank [4, 5] and Foresman [6] have shown that, in rats, a synthetic dressing has the same effect as a full thickness skin graft in reducing open wound contraction in the short term. How the synthetic dressing achieved this remains unclear. It is possible that it acted as a splint or it may be that, by allowing wound healing to take place in a moist environment, the dressing reduced the stimulus to wound contraction. We decided to see if these dressings had a similar effect in humans. We accept that split skin graft donor sites are not full thickness wounds but our study shows that these wounds do, in fact, contract in the short term. Other workers have noted that even in partial thickness wounds there is increased dermal collagen metabolism which can be influenced by locally produced growth factors [3, 10] indicating that wound contraction (as in this study) is possible in partial thickness wounds. The perhaps surprisingly high figure of a 20% contraction of the control wounds is interesting. These donor sites were all surrounded by normal, lax skin and thus any physical forces opposing contraction would be minimal [15]. This figure may vary depending on the position of the donor site.

If the dressings were reducing wound contraction by their splintage action only, then the conventionally dressed donor sites should contract minimally as these dressings become hard and remain adherent to the wound until it epithelialises. The synthetic dressing used in this study had a minimal splintage effect so the reduction of contraction must have been a result of other factors. Recent work in rats has shown that the wound under a split skin graft overed with a synthetic dressing reacts as though it is covered with a full thickness graft. Preliminary work indicates that dressing a split skin graft in this way (for only one week) reduces the underlying myofibroblast cell populations [9]. It is possible that the reduction of human donor site contraction may be a result of the synthetic dressing's effect either on the underlying myofibroblast cell population or its effect on wound epithelialisation or both. The moist environment produced by the synthetic dressing is not the whole answer to how it reduces underlying wound contraction [2, 6]. It may be that the synthetic dressing influences the accumulation of factors in the locality of the wound which, in turn, help to increase epithelialisation and reduce wound contraction [2].

Maximum wound contraction occurs in rats at 2–4 weeks [13, 14]. This coincided with the clinical practice of removing donor site dressings so measurements were taken at that time, it would have been interesting to follow up these patients over a longer period of time to see if this initial difference in contraction remains. This was not possible as the majority of these patients lived a considerable distance from the hospital and regular follow-up was difficult.

It would seem that partial thickness wounds, in humans, do contract. This contraction can be measured as early as 14–17 days after wounding and can be influenced in humans as it is in rats by the material used to dress the wound. This ability would be useful in a burns unit as burns of varying depths with and without skin grafts could be covered with these dressings with the hope of reducing the long-term contracture problems associated with these injuries. Although synthetic dress-

ings have been used as a temporary dressing in superficial burns [12] no studies have been reported where their long-term effect on wound contraction has been investigated. Such studies are currently in progress.

Acknowledgements. We would like to thank Mr. A.M. Morris for allowing us to include his patients in this study.

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Eur J Plast Surg (1990) 13:192–197

European Journal of Plastic Surgery

A new experimental model of lymphedema in rats' hind lower legs

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Summary. In this study, a simple and reliable experimental model for lymphedema is described. In the middle portion of albino rats' hind lower leg, leaving the saphenous vessels and the accompanying lymphatics intact, all other soft tissues are incised circularly. After retraction of the cut ends of muscles, the skin edge of the ventral side is sutured to that of the dorsal side to envelope the muscles of both cut ends and the saphenous vessels and lymphatics. Resection of lymphatics or circular strip of soft tissues is not performed. In 48 of 50 rats' legs, measurements were made of leg circumference and the diameter of the lymphatics; in addition changes of lymphatic contractility were observed. These measurements, observations and histopathological studies showed a consistent picture of lymphedema. The clinical implication of this observation is that a small number of lymphaticovenous anastomoses will not correct lymphedema and that the quality of the results will correlate with the number of anastomoses. In studying the findings of experimental lymphedema, some other clinical phenomena are explained and modifications of the operative procedure of microlymphaticovenous anastomosis for treating lymphedema are suggested.

Key words: Lymphedema – Lymphaticovenous anastomosis – Experimental model

In order to study the mechanism of lymphedema formation and its therapeutic implications make it desirable to produce an experimental model for lymphedema. It is very difficult to establish models of lymphedema because of the regeneration of lymphatics, in this way severed lymphatic vessels are reconstituted to functional or anatomic perfection in most instances. In 1968, Olszewski et al. [14] excised a 2 cm circular strip of skin and subcutaneous tissue in the dog thigh and resected a 4 cm length of femoral lymphatics; a 1 cm strip of periosteum was excised, and the popliteal lymph node was removed. The skin was sutured to muscle and allowed to heal by granulation. This technique resulted in temporary swelling in all dogs, but chronic lymphedema developed in only eight of 23 dogs. In 1974, Clodius et al. [2] made an experimental dog model by a circular excision of soft tissue including all lymphatics for a distance of 4 cm in the thigh followed by insertion of a synthetic implant (polyurethane foam – Etheron) to prevent lymphatic regeneration.

In this study, a new experimental model of lymphedema in rats' hind legs is described. In this model, resection of lymphatics and a circular strip of soft tissues was not performed. The technique is simple; the method is inexpensive and has a high success rate. In 48 of 50 rats' hind lower legs, lymphedema could be demonstrated over six months of observation.

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Material and methods

Fifty albino rats weighing 200 to 300 g were used. The rats were anesthetized with an intraperitoneal injection of 3% pentobarbital sodium (30 mg/kg). The left hind leg was operated on; the right hind leg served as a control. For visualization of lymphatics, 1% methylene blue (0.05 ml) was injected into the sole of the foot. Due to the uptake of the dye, two to three major lymphatic channels around the saphenous artery and vein in the middle portion of the rat's lower leg could be clearly visualized. The saphenous vessels along with the lymphatics were carefully dissected and preserved. All other soft tissues in the middle portion of the lower leg were incised circularly (Fig. 1). After retraction of the cut ends of the muscles, the skin edge of the ventral side was sutured to that of the dorsal side to wrap the muscles of both cut ends and the continuing saphenous vessels and lymphatics (Fig. 2). No resection of lymphatics or a circular strip of soft tissue was performed. In the model, the major lymphatics around the saphenous vessels were preserved, but the formation of lympholymphatic anastomoses of other severed lymphatics and collateral lymph flow through the gap between the cut ends were prevented, otherwise, all wounds were closed and allowed to heal without inflammation and granulation.

After the operation, two rats died of anesthesia. The surviving

Eur J Plast Surg (1989) 12:258–260

European Journal of Plastic Surgery

A Simple and Reliable Method for Rapidly Harvesting Split Skin Grafts in Rats

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Summary. A technique is presented which allows the rapid precise harvesting of split-skin grafts in rats. This technique uses the skin which would have been discarded in the formation of an open wound as a donor site thus reducing the overall assault on the animal.

Key words: Harvesting method – Split-skin grafts – Rats

The rat is a good animal model regularly used in wound healing studies. The presence of a panniculus carnosus, however allows considerable skin mobility which makes split skin graft harvesting difficult. The use of a battery or electric powered dermatome allows repeatable precise graft harvesting but this must be used with an anchoring appliance. Previous reports have suggested the use of a clamp, but the techniques described require an assistant or genetically similar donor rats [2–7]. These methods are time consuming and can only be used by a single operator with difficulty.

This paper presents a technique which:

a) requires no assistant
b) uses the skin to be excised in the formation of an open wound as a donor site thus eliminating the need for donor rats or separate wounds on the same animal
c) requires one custom made sheet of metal which takes a few seconds to position to allow easy graft harvesting
d) allows rapid graft harvesting, on average the harvesting and application of 4 grafts on one rat took 20–30 min which negated the need for per-operative top-up anaesthesia
e) is reproducable. This technique has been used to harvest 800 similar skin grafts.

Method

The anaesthetised rat is shaved and depillated in the standard manner. The area of skin to be harvested is marked with ink. In this instance 2×2 cm open wounds were to be covered with split skin grafts harvested from the excised skin. Two incisions were made in the opposite sides of the square through the panniculus carnosus. The plane under this structure was mobilised with scissors and the flat steel plate inserted (Fig. 1).

Experience showed that if the plate was 50% longer than the area to be harvested (in this case 3 cm) the correct amount of tension could be easily applied to allow rapid precise graft harvesting. The incisions could be minimally lengthened to permit easier plate insertion.

The skin immediately cranial and caudal to the plate is grasped between the index finger and thumb of the non-dominant hand, and by approximating these digits sufficient tension can be applied to flatten the skin against the metal block. This tension usually resulted in the uncut marked sides of the square moving to the edge of the plate (Fig. 2).

The skin is then lubricated with a little mineral oil and harvested with an electric dermatome. If the cutting head is 50% longer than the width of the skin graft the side of the blade does not impede or disrupt the harvesting process by cutting into the skin (Fig. 3).

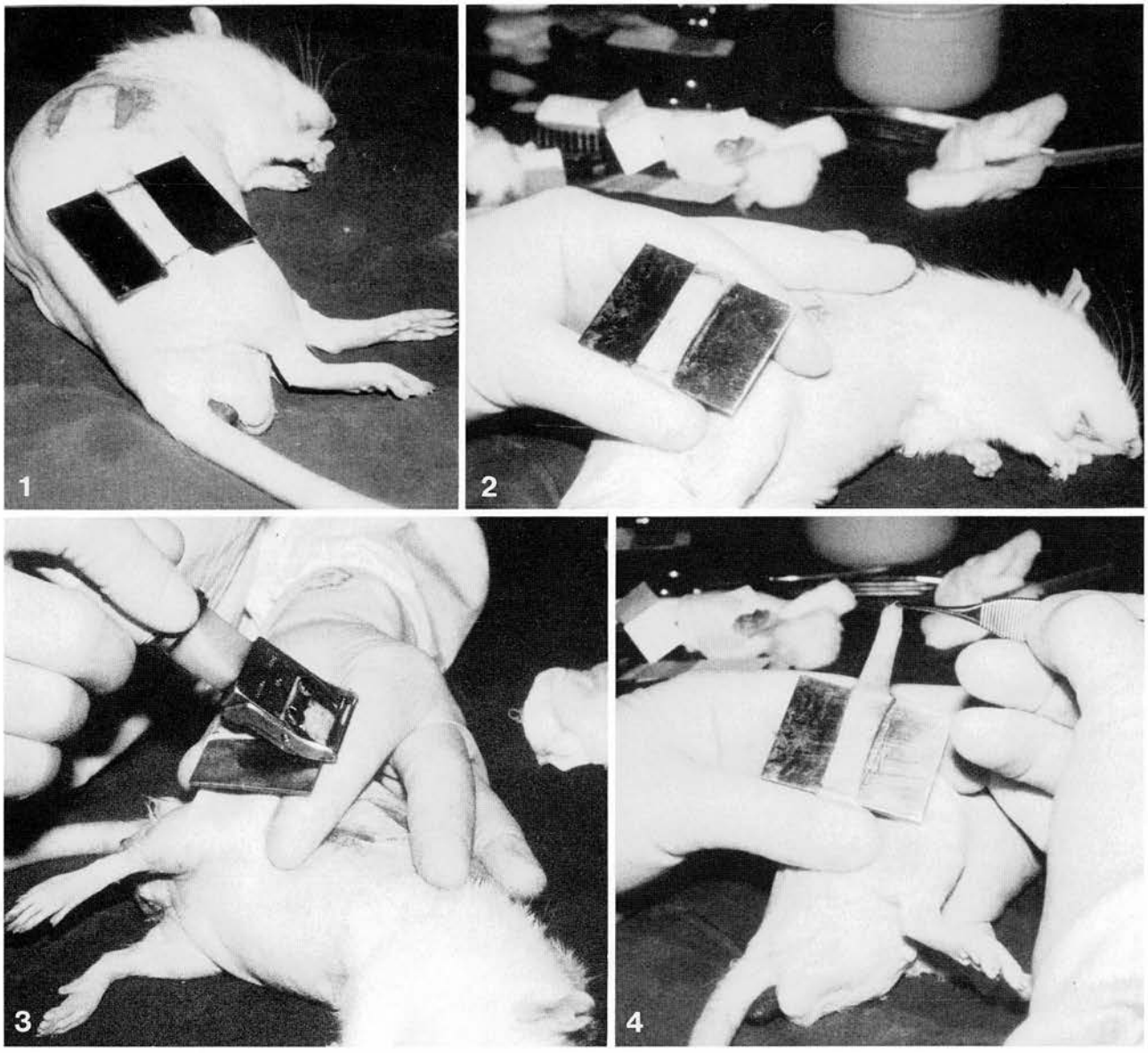
Once the predetermined amount of skin is harvested (to the previously marked fourth side of the square in this case), the dermatome is stopped and withdrawn. This reduces the risk of the cutting head damaging the newly harvested graft. When the skin is removed from the dermatome the attached edge is cut freeing the graft (Fig. 4). The underlying dermis is then excised allowing the easy removal of the metal plate, revealing an open wound ready for skin grafting.

Eight hundred skin grafts were harvested using this technique and sample measurements were made on 50 grafts using an electric micrometer (Model Mitutoyo Digimatic Indicator range 0.002–30 mm).

Of the 50 sample grafts, mean graft thickness was 0.229 mm with a standard error of the mean of 0.015 mm indicating the reliability of the technique to produce grafts of similar thickness.

Discussion

Techniques described in the literature require either genetically similar donor rats or appliances



Figs. 1–4

designed to anchor the skin of the rat with clamps or stay sutures. These techniques are time consuming and/or require an assistant.

The technique presented allows the rapid, precise harvesting of split skin grafts in the rat by a solo operator. The skin to be discarded in the formation of an open wound is used as the donor site for the split skin graft. The thickness of the split skin graft can be varied by altering the setting on the electric dermatome. The tension on the skin during the grafting process is controlled by aligning the edges of the square with the edge of the underlying metal plate thus ensuring all grafts are harvested using the same amount of tension. The technique was quickly mastered and there were only 5 failures which were attributed to a blunt knife chewing up the graft.

The increase in size produced by stretching the skin was lost when the skin graft underwent primary, physiological contraction [1] immediately after harvesting, returning to its original size.

In summary this technique, which allows precise skin grafts to be rapidly harvested by one operator, is to be recomended when wounds deep to the panniculus carnosus are being formed. As the split skin grafts are being harvested from skin which otherwise would have been discarded in the formation of the open wound the need for donor

rats is eliminated and the overall assault on the animal is reduced.

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Forthcoming Meetings & Events

European Journal of Plastic Surgery

January

January 3–5, 1990 – *Los Angeles, USA*
Third International Interdisciplinary Research Conference on Fundamentals of Bone Growth: Methodology and Applications
Information: Drs. A.D. Dixon or B.G. Sarnat, Schools of Dentistry and Medicine, 63-090 CHS, University of California Los Angeles (UCLA), Los Angeles, CA 90024, USA.

January 14–20, 1990 – *Vail, Colorado, USA*
Update – Craniosynostosis
Information: Christine Rogers, MD, or Lawrence Ketch, MD, phone (303) 270-4542.

January 25–February 8, 1990 – *Tanzania*
Third Annual East African Ophthalmic Plastic and Reconstructive Surgery Symposium
Information: St.L. Bosniak, MD, 300 Central Park West, New York, NY 10024, USA.

February

February 7–10, 1990 – *Los Angeles, USA*
Cleft Lip/Cleft Palate/Maxillofacial Symposium
Information: Ms. C. Gallagher, ASPRS Executive Office, 444 East Algonquin Road, Arlington Heights, IL 60005, USA.

February 11–16, 1990 –*Hawaii*
20th Biennial Surgical Congress – Pan Pacific Surgical Association
Information: John Smith, MD, Secretary, P.P.S.A., 733 Bishop Street, Suite 1910, Honolulu, HI 96813, USA.

March

March 14–16, 1990 – *Cairo, Egypt*
Annual Meeting of the Egyptian Society of Plastic and Reconstructive Surgeons
Information: Dr. M. Kadry, Secretary, ESPRS, "Dal El Hekmah", 42, Kasr El-Eini Street, Cairo, Egypt.

March 16–17, 1990 – *Winnipeg, Manitoba, Canada*
Early Versus Late Palatal Closure
Information: P.T. Alexander, Ph.D., Director, Department of Communication Disorders, Health Sciences Centre, FE207, 685 William Avenue, Winnipeg, Manitoba R3E 0Z2, Canada.

March 28–31, 1990 – *New York, N.Y., USA*
International Symposium on the Interdisciplinary Management of Complex Craniofacial Disorders
Information: Dr. C. Hall, Dr. J. Goodrich, or Dr. R. Shprintzen, Center for Craniofacial Disorders, Montefiore Medical Center, 111 East 210 Street, Bronx, NY 10467, USA.

April

April 2–4, 1990 – *Barcelona, Spain*
XVth Course on Temporal Bone Dissection
Information: P. Clarós, MD, PhD, ENT Department, San Juan de Dios Hospital, Carretera de Esplugas s/n, E-08034 Barcelona, Spain.

May

May 25–26, 1990 – *Bad Homburg, FRG*
Traumatology in Childhood
Information: Dr. B. Hoffmeister, Arbeitsgemeinschaft für Kieferchirurgie, Arnold-Heller-Straße 16, D-2300 Kiel 1.

May 25–31, 1990 – *Hannover, FRG*
The Third European Flap Course
Information: Dr. E. Schaller, Secretary, Clinic of Plastic, Hand and Reconstructive Surgery, Medical School Hanover, Podbielskistraße 380, D-3000 Hannover 51, Federal Republic of Germany.

Originals

European Journal of Plastic Surgery

Eur J Plast Surg (1990) 13:97–100

Alteration of split skin graft contraction with a synthetic dressing

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Summary. In a rat model, covering a split skin graft with a synthetic dressing for only one week alters the subsequent contraction characteristics of the underlying wound so that the graft increases in size in a manner similar to a full thickness skin graft.

Key words: Split skin grafts – Synthetic dressing – Graft contraction alteration

In open wounds the normal healing process involves wound contraction [14], the control of which would be a useful weapon in the surgeon's armamentarium.

Covering a wound with a skin graft reduces contraction. Clinically and experimentally, wounds covered with full thickness skin grafts contract minimally but those covered with split skin grafts can contract by up to 60% [8, 12, 15]. The presence of dermis was felt to be important [3], but a synthetic dressing has been shown to have the same effect, in the short term, as a full thickness skin graft in reducing contraction in open wounds [6, 7]. Reduction of moisture loss and adherence of the dressing may be important factors in reducing wound contraction [5]. If a synthetic dressing has this effect on contraction of open wounds, what effect, if any, does it have on wounds covered with split skin grafts?

A study was therefore set up to observe both surface area and moisture loss from split skin grafted wounds, one on each flank in rats, one side covered with a synthetic dressing, the other side acting as control.

Materials and methods

Animal model

Forty immature male Sprague Dawley rats (mean weight 384.4 g ± 5.3 g standard error of the mean) were used in this experiment. All rats were individually housed in cages kept in an environment whose temperature and humidity were carefully controlled.

One full thickness wound (2 × 2 cm including panniculus carnosus) was created on each flank after depilation. The excised skin was used to harvest a split skin graft (mean thickness 0.229 mm; SEM 0.012 mm) with an electric dermatome (Servomed UK). Graft thickness was checked with an electric micrometer and sample biopsies were taken of the grafts and underlying tissue to histologically confirm graft thickness and the similarity of both sides. The grafts were then applied to the defects and secured with sutures. On one side the graft was covered with Opsite cut to shape, the other side acting as a control. A protocol was devised so that the rats would be studied in 5 groups of 8, each group having Opsite in situ for 1, 2, 3, 4 or 5 weeks. Measurements were then taken.

Water loss

Water loss or moisture vapour transmission (MVT) was measured with an evaporimeter. Measurements were taken from normal skin and open wounds to compare with published data [5]. Readings were also taken from the split skin grafts covered with Opsite and those acting as controls. MVT was measured when the dressing was applied, when it was removed, the day following removal and thereafter at regular intervals up to the end of the study.

Surface area

Tracings on acetate sheets were made of each skin graft at operation and at regular predetermined intervals up to 15 weeks postoperatively. These tracings were transferred to a computer and the surface area was measured and expressed in square millimeters. The mean values and standard error of the mean (SEM) per group were then calculated.

Dressings

Once a skin graft was covered with Opsite, both grafts on the animal were treated identically. A cotton wool ball was held over the grafts with 4 ties over sutures. The rat was then placed in a protective towelling dressing and a restrictive vest applied to the anterior aspect of the thorax [13]. Regular inspection occurred and any disturbance of the dressing repaired. This technique was well tolerated by the animals and few repairs were necessary. After 1 week, under gaseous anaesthesia, the ties over dressings were removed and wound measurements taken according to the protocol. The rats were then redressed with the towelling dressing and the protective vest. These dressings were later removed under anaesthesia for further measurements according to the protocol.

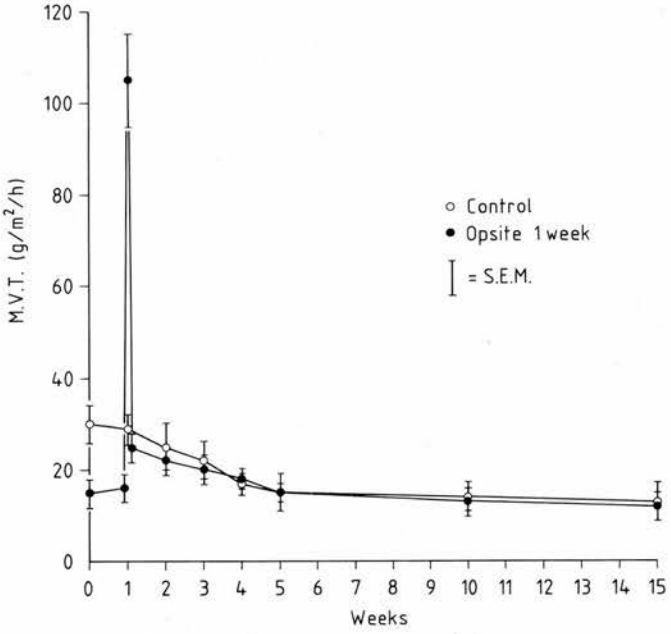

Fig. 1. Water loss or moisture vapour transmission

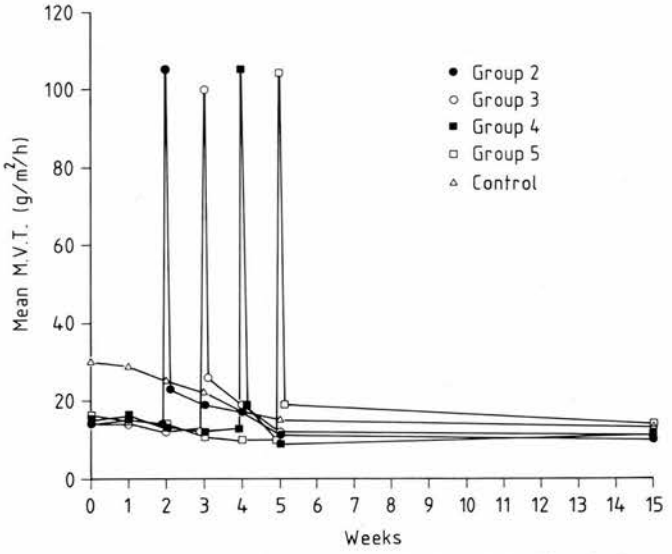

Fig. 2. Water loss groups 2, 3, 4 and 5. SEM excluded for clarity

Results

Growth

Mean rat weight fell by 13.8% in the first postoperative week and thereafter weight gain took place at a mean rate of 4.7% per week until the end of the study when mean rat weight was 582.4 g.

Failures

The study was statistically designed to accommodate a 25% failure rate. In fact only 3 rats were excluded from the study (7.5%). These were due to graft failures associated with a seroma (1 rat), haematoma (1 rat), and autocanabalisation (1 rat). This indicated how well the dressings were tolerated by the animals.

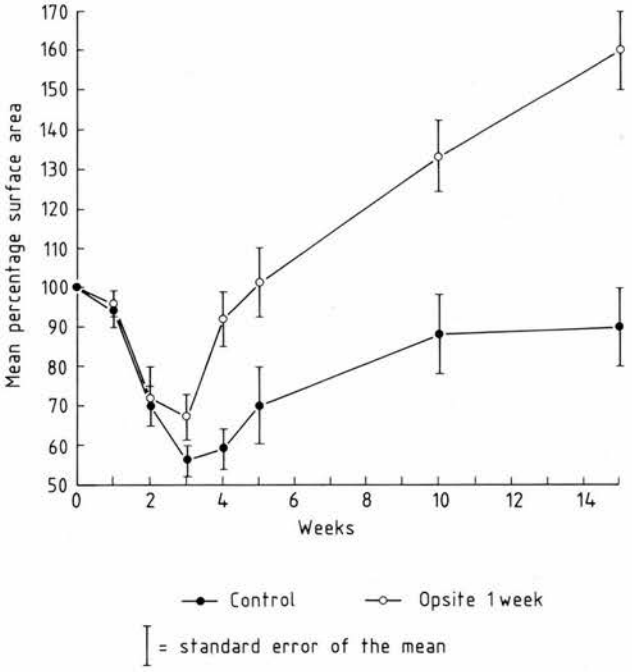

Fig. 3. Change in surface area group 1

Water loss

Moisture vapour transmission (MVT) from open wounds and normal skin had respective values 60 g/m^2/h (GMH) and 5 GMH. This compared well with previously published data [5].

Covering a split skin graft with a synthetic dressing enormously increased the humidity of the wound healing environment. When this dressing was removed there was a massive surge in moisture vapour transmission which returned to the control group values after 1 day (Fig. 1). Readings taken the day following dressing removal were not significantly different from values recorded before dressing removal. This phenomenon occurred in all groups and there was no statistical difference in the peak readings recorded in the first few minutes following dressing removal (Fig. 2).

Surface area

Controls. The split graft controls in this experiment behaved in a manner similar to published data [1, 3, 12, 15]. There was an initial reduction to 56% of the original size at week 7 and thereafter a slow increase in size to 90% at week 15 (Fig. 3).

Opsite. Covering a split skin graft with Opsite for only 1 week resulted in the greatest increase in surface area (Fig. 3). In this group the difference between the control

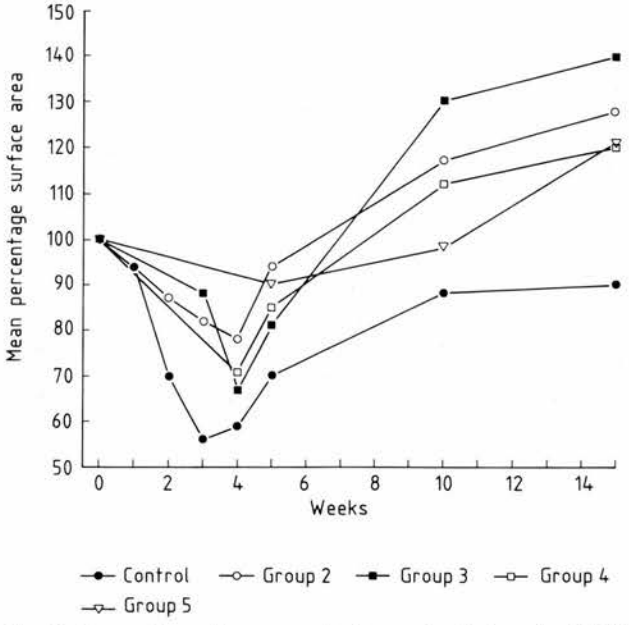

Fig. 4. Change in surface area; Opsite on for 2–5 weeks. SEM excluded for clarity

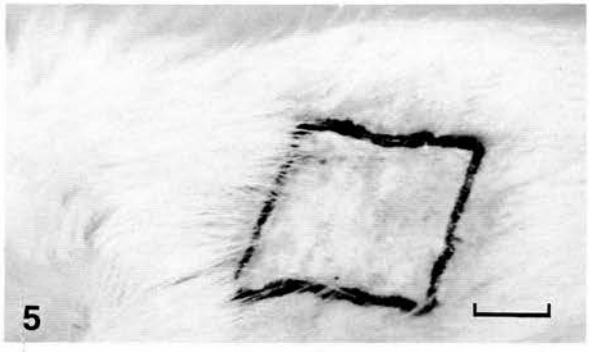

Fig. 5. Covered with Opsite for one week

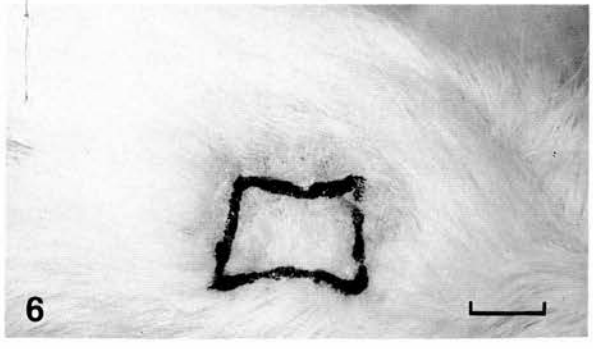

Fig. 6. Control

grafts and the Opsite covered grafts became statistically significant (no overlap in the standard error of mean between the 2 populations) 2 weeks after Opsite removal. This difference increased until at week 15 there was a 70% difference between the Opsite and control groups (Figs. 5 and 6). Covering a graft with a synthetic dressing for longer than 1 week did not result in any greater increase in subsequent surface area values (Fig. 4).

Discussion

Skin grafting is a routine technique used by plastic surgeons to reconstruct defects resulting usually from trauma, skin tumour excision and burns. Split skin grafts are used in preference to full thickness skin grafts for many reasons [2, 8]. Self healing donor sites allowing large graft harvesting, and better take, are the main advantages but one major disadvantage is that wounds covered with split skin grafts contract to a greater degree than wounds covered with full thickness skin grafts. In order to counteract this tendency, regimes incorporating extensive splintage, pressure garment use, physiotherapy and perhaps even secondary corrective surgery are necessary. This study shows that in rats, the application of a synthetic dressing to a split skin graft allows this phase of wound healing to take place in a very moist environment. Split skin grafts treated in this way contract less and progress to a greater size than the untreated controls. How synthetic dressings achieve this is unclear, but covered wounds appear to act as if they were covered by full thickness skin grafts. Frank [6, 7] who noted a similar temporary effect of synthetic dressings on open wounds concluded that intimate dressing adherence is necessary to achieve this effect. This observation is endorsed by Foresman, who showed different rates of wound contraction between different dressings [5]. These dressings adhered to the wound in different ways, the ones with greater adherence showing a greater reduction in contraction of the open wounds.

In this study, the synthetic dressing was applied to the epidermal aspect of a split skin graft and it was thus easier to obtain more of an intimate adherence to the skin graft than to an open wound. It can be assumed that intimate adherence of a synthetic dressing not only reduces water loss but allows gaseous exchange and it is interesting to note that the synthetic dressing maintains its contraction reducing effect even when a split skin graft is sandwiched between the dressing and the open wound. In this study, however, the effect the synthetic dressing had, only began to statistically manifest on surface area figures several weeks after dressing removal whereas open wound contraction was affected only when the dressing was in situ. When Opsite was removed after 1 week, this group showed the largest surface area values at the end of the study, some 14 weeks after the dressing was removed. This implies that the effect the dressing has is in the first few hours [4] or days of the wound healing process and in order for this effect to continue after the dressing removal it would seem logical that a cellular component of the wound healing process is affected. Myofibroblasts are now considered to be the cells responsible for wound contraction [11] and Rudolph [9, 16] has shown that the myofibroblast cell populations are different in wounds covered with full thickness skin grafts compared to wounds covered with split skin grafts. Further studies are in progress to identify and quantify the myofibroblast populations under split grafts with and without Opsite dressings. Preliminary findings [10] indicate that myofibroblast cell orientation beneath the skin graft may play an important

role in the different rates of contraction in both groups. From this study it can be concluded that in the rat the application of a synthetic dressing to a split skin graft in the early phase of wound healing allows the split skin graft to increase in size in a manner similar to a full thickness skin graft.

Acknowledgements. I would like to thank Smith & Nephew Research Division for the use of their facilities and encouragement without which this research would not have been possible.

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